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# **XXI REUNIÓN DE LA SOCIEDAD ESPAÑOLA DE QUÍMICA ANALÍTICA**

***Valencia, 5-7 de Septiembre de 2017***

**Libro de resúmenes**



**SEQA  
2017**

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ISBN: 978-84-697-4966-1

In Memoriam Guillermo Ramis Ramos (1950-2017)







**BIENVENIDA DEL COMITÉ CIENTÍFICO Y DEL COMITÉ ORGANIZADOR  
A LA XXI REUNIÓN DE LA  
SOCIEDAD ESPAÑOLA DE QUÍMICA ANALÍTICA (SEQA)**

En nombre de todos los compañeros que han trabajado por diseñar y preparar la XXI Reunión SEQA en la Universitat de València, os queremos dar la más cordial y afectuosa bienvenida. De nada sirve nuestro esfuerzo, si no conseguimos alcanzar vuestra voluntad para participar y contribuir a la reunión de los químicos analíticos españoles. No os sorprenda por tanto que nuestra bienvenida vaya cargada de inmenso agradecimiento por vuestra presencia.

Gracias a vuestras contribuciones y a las conferencias invitadas, ofrecemos un programa científico de calidad; la eficiencia y amabilidad de la organización la vais a comprobar en los próximos días, y poco más podemos añadir ahora. En ese poco, queremos insistir en la relevancia de la Reunión SEQA porque aspira a congrega a una comunidad científica que comparte inquietudes e ilusiones y que se enfrenta a un dinamismo y competitividad científica y docente sin precedentes. Y ante una mayor y progresiva especialización científica y curricular, aparentemente imprescindibles para el desarrollo profesional, la SEQA ofrece un programa policrómico, un variado recorrido por conocimiento y técnica químico analítica. Huelga decir que un programa de dos días no puede ofrecer más que un flash del estado del arte, pero aún así hemos aspirado a ofrecer una visión del presente, evolución y crecimiento de la Química Analítica actual.

Y en esta visión de conjunto, incidimos en la docencia de la Química Analítica con la celebración de la III Jornada de Innovación Docente. El Grupo de Especiación ha querido también sumarse a esta iniciativa por aunar esfuerzos y ha celebrado una Jornada centrada en el arsénico como diana de especiación y regulación en alimentos. Ambas Jornadas han precedido a esta XXI Reunión y han calentado motores para un encuentro que viene además acompañado de diversas y variadas citas. Entre ellas, la entrega de los dos premios Miguel Valcárcel, la entrega de los premios de la XXI Reunión, la Asamblea General de la Sociedad con la elección de una nueva presidencia y un buen número de actividades sociales que nos aventuramos a imaginar, dejarán una huella inolvidable en nuestra pequeña gran historia de encuentros.

Y no queremos terminar esta bienvenida sin agradecer a todas las instituciones y entidades que han hecho posible nuestra Reunión. Nuestro agradecimiento a la Universitat de València, a la Facultat de Filologia, Traducció i Comunicació y, sobre todo, a los patrocinadores que con sus aportaciones han contribuido a que esta XXI Reunión SEQA sea una realidad.

Elena Domínguez  
Presidenta SEQA

Salvador Garrigues  
Presidente del Comité Organizador



## **PROGRAMA**





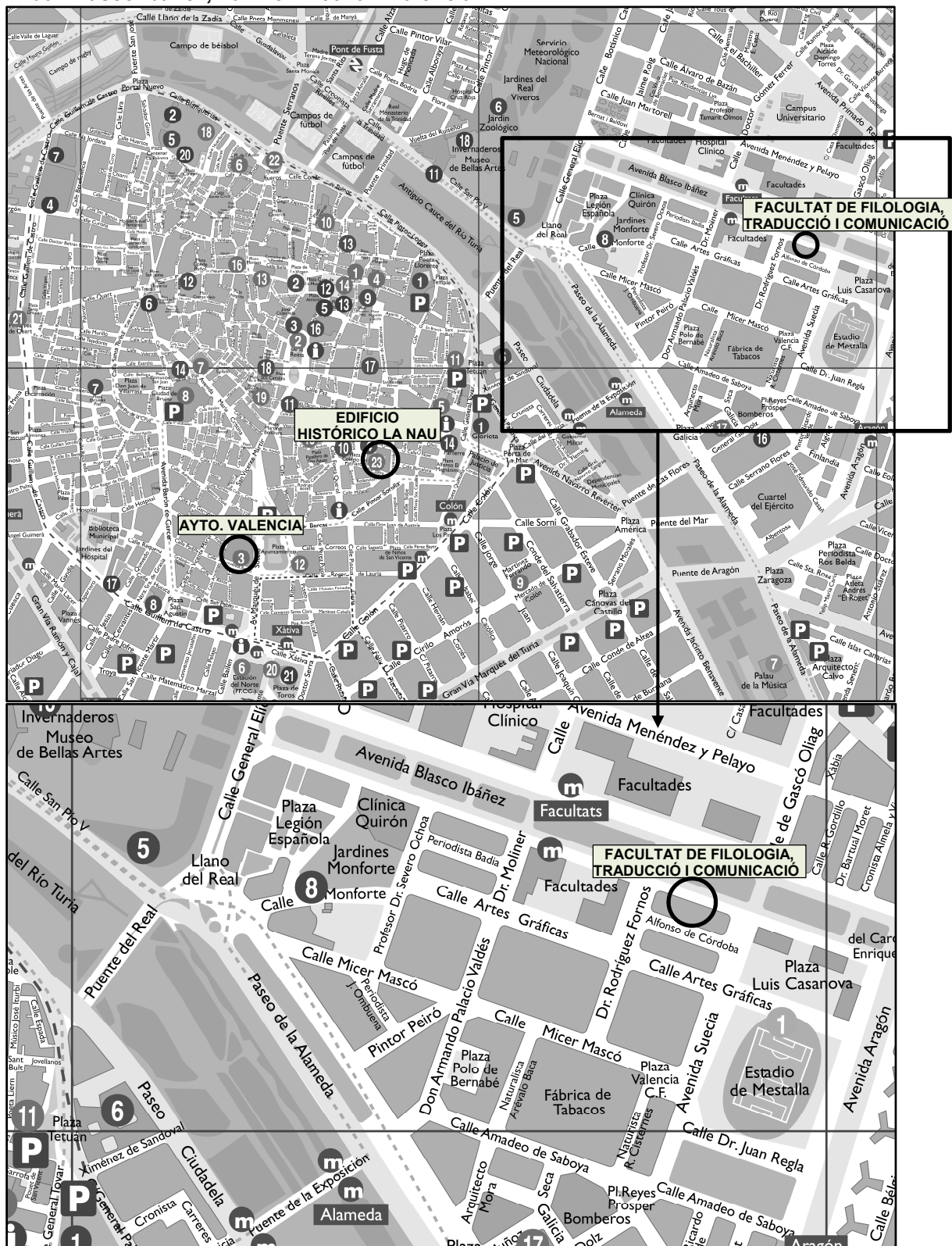
**Comité Científico:**

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Lugar de celebración:  
 Facultat de Filologia, Traducció i Comunicació.  
 Avda. Blasco Ibáñez, núm. 32. 46010 – València



## XXI REUNIÓN DE LA SOCIEDAD ESPAÑOLA DE QUÍMICA ANALÍTICA

### PROGRAMA DE LA REUNIÓN.

<b>MARTES 5 DE SEPTIEMBRE 2017</b>	
<b>16:00-19:00</b>	<b>Entrega de documentación.</b> Facultat de Filologia, Traducció i Comunicació. Avda. Blasco Ibáñez, núm. 32, 46010 - València
<b>20:30-22:30</b>	<b>Recepción de bienvenida.</b> Edificio Histórico La Nau. Carrer de la Universitat, 2, 46003 - València

<b>MIÉRCOLES 6 DE SEPTIEMBRE 2017</b>	
<b>08:30-09:00</b>	<b>Recogida de documentación / Colocación de posters</b>
<b>09:00-09:30</b>	<b>Ceremonia apertura XXI Reunión SEQA</b> <i>Elena Domínguez Cañas (Presidenta de la SEQA)</i> <i>Salvador Garrigues Mateo (Chairman de las XXI Reunión SEQA)</i>
<b>09:30-10:15</b>	<b><u>Conferencia inaugural:</u></b> <b>Innovations in Mass Spectrometry Platform Technologies for Epithelial Ovarian Cancer Research</b> <i>David C. Muddiman; NC State University, USA.</i> <i>Moderador: José Luís Gómez Ariza</i>
<b>10:15-11:00</b>	<b><u>Sesión comunicaciones orales:</u></b> <i>Moderador: M<sup>a</sup> Teresa Tena y Alberto Chisvert</i> <b>OR-01 Sheathless capillary electrophoresis-mass spectrometry in the metabolic profiling of biomass-restricted samples. Metabolomic analysis of a mouse model of polycystic kidney disease.</b> <i>E. Sánchez-López, G. S. M. Kammeijer, A. L. Crego, M. L. Marina, R. Ramautar, D. J. M. Peters, O. A. Mayboroda.</i> <b>OR-02 Estrategias analíticas basadas en espectrometría de masas en la búsqueda de nuevos biomarcadores del cáncer de mama: la MMP-11.</b> <i>M. L. Fernández-Sánchez, R. González de Vega, P. Abásolo Linares, J. Pisonero, N. Ediro, F. Vizoso, U. Karst and A. Sanz Medel.</i> <b>OR-03 Element-tagged immunoassays with ICP-MS detection for quantification of some iron-regulatory proteins in breast cancer cells: on the search for cancer biomarkers.</b> <i>J. Alonso García, E. Blanco-González, E. Añón Álvarez, M. Montes-Bayón.</i>
<b>11:00-11:25</b>	<b>Café / Posters</b>
<b>11:25-12:00</b>	<b><u>Conferencia invitada:</u></b> <b>GCxGC-TOF-MS – A Powerful Technique for Environmental Studies</b> <i>Lourdes Ramos; Instituto de Química Orgánica General, CSIC, Madrid, Spain.</i> <i>Moderador: Pilar Bermejo</i>
<b>12:00-12:10</b>	<b><u>Presentación Técnica - Patrocinador Platino:</u></b> <b>PT-01 Analysis of mycotoxins in food matrices.</b> <i>Jaume C. Morales - Agilent Technologies</i>



12:10-13:25	<p><b>Sesión comunicaciones orales:</b>  <u>Moderadora: Antonia Garrido y Encarnación Rodríguez</u></p> <p><b>OR-04 Screening of new psychoactive substances in urine samples by high resolution mass spectrometry.</b>  <u>Noelia Salgueiro-Gonzalez; Sara Castiglioni; Emma Gracia-Lor; Nikolaos I. Rousis; Lubertus Bijlsma; Alberto Celma; Felix Hernandez; Ettore Zuccato.</u></p> <p><b>OR-05 LC-QTOF MS/MS analysis of changes in the composition of the polar fraction of persian lime (<i>Citrus latifolia</i>) during fruit growth.</b>  <u>C.A. Ledesma-Escobar, F. Priego-Capote, V.J. Robles-Olvera, M.D. Luque de Castro.</u></p> <p><b>OR-06 Novel and versatile microextraction approaches based on dispersion of magnetic materials coating stir bars.</b>  <u>Juan L. Benedé, Alberto Chisvert, Dimosthenis L. Giokas, Jared L. Anderson, Amparo Salvador.</u></p> <p><b>OR-07 Extracción mediante electromembrana integrada en un dispositivo microfluídico para la determinación de fluoroquinolonas en aguas medioambientales.</b>  <u>Elia Santigosa Murillo, Jordi Coello, María Ramos Payán.</u></p> <p><b>OR-08 Nanometrología analítica: hacia la resolución de problemas complejos.</b>  <u>F. Laborda, I. Abad-Alvaro, E. Bolea, G. Cepriá, C. Cubel, M.T. Gómez-Cotín, M.S. Jiménez, J. Pérez-Arantegui, J.C. Vidal, J.R. Castillo, V. Taboada, A. Moreda, MC. Barciela, P. Bermejo.</u></p>
13:25-15:30	<p><b>Sesión de posters – Comida</b></p>
15:30-16:15	<p><b>Conferencia invitada:</b>  <b>Programming flow in capillary microfluidics: from concepts to assays</b>  <b>Emmanuel Delamarche; IBM, Zurich Research Laboratory, Switzerland.</b>  <u>Moderadora: Ángel Ríos</u></p>
16:15-17:00	<p><b>Sesión comunicaciones orales:</b>  <u>Moderadora: Elisa Blanco y José Manuel Herrero</u></p> <p><b>OR-09 Detection of B-type natriuretic peptide (BNP) at screen printed carbon electrodes modified with gold nanoparticles grafted through aryl diazonium salt chemistry.</b>  <u>V. Serafín, R.M. Torrente-Rodríguez, M. Batlle, P. García de Frutos, S. Campuzano, P. Yáñez-Sedeño, J.M. Pingarrón.</u></p> <p><b>OR-10 Direct electrochemical tests on urine as non-invasive strategy for detecting urothelial carcinoma.</b>  <u>Antonio Doménech-Carbó, José Luís Pontones, Clara Doménech-Casasús, Josefina Artés, Sara Villaroya, David Ramos.</u></p> <p><b>OR-11 Evaluation of multivariate calibration of isobaric compounds in a FIA-MS/MS context.</b>  <u>Ana María Casas-Ferreira, Encarnación Rodríguez-Gonzalo, Bernardo Moreno-Cordero, José Luís Pérez-Pavón.</u></p>
17:00-17:25	<p><b>Café / Posters</b></p>
17:25-18:30	<p><b>Presentación / Discusión Posters</b></p>
18:30-19:30	<p><b>Asamblea General SEQA / Votación</b></p>
20:30-23:00	<p><b>Recepción en el Ayuntamiento de Valencia y visita al centro histórico.</b></p>

<b>JUEVES 7 DE SEPTIEMBRE 2017</b>	
09:30-10:15	<b>Conferencia invitada:</b> <b>Nano-Liquid Chromatography: Main Features and Potentiality in Separation Science.</b> <b>Salvatore Fanali;</b> <i>Institute of Chemical Methodologies. CNR, Italy.</i> Modera: <u>M<sup>a</sup> Luisa Marina</u>
10:15-11:00	<b>Sesión comunicaciones orales:</b> Modera: <u>María Pedrero y Sergio Armenta</u> <b>OR-12 Sample introduction systems involving low sample consumption for multi-element determinations in serum by ICP-MS.</b> <u>María del Pilar Chantada-Vázquez, Paloma Herbello-Hermelo, Jorge Moreda-Piñeiro, Pilar Bermejo-Barrera, Antonio Moreda-Piñeiro.</u> <b>OR-13 Measuring hold-up volume and phase ratios in HILIC.</b> <u>Xavier Subirats, Althea Justicia, Martí Rosés.</u> <b>OR-14 Simultaneous determination of additives in food products by digital image analysis.</b> <u>Maider Vidal, Rosa García-Arrona, Ane Bordagaray, Miren Ostra, Gorka Albizu.</u>
11:00-11:25	<b>Café/Posters</b>
11:25-12:00	<b>Conferencia invitada:</b> <b>La Química Analítica frente a los retos de la globalización.</b> <b>Ángel Maquieira;</b> <i>IDM, Departamento de Química, Universitat Politècnica de València, Spain.</i> Modera: <u>Miguel de la Guardia</u>
12:00-12:10	<b>Presentación Técnica - Patrocinador Platino:</b> <b>PT-02 Nuevo ICP-MS "NEXION 2000": cualquier matriz - cualquier interferencia - cualquier tamaño de partícula.</b> <u>Xavier Milá Niubó – Perkin Elmer España S.L</u>
12:10-13:25	<b>Sesión comunicaciones orales:</b> Modera: <u>Manuel Hernández Córdoba y Amparo Salvador</u> <b>OR-15 Antimony migration studies in fruit juices stored in polyethylene terephthalate bottles.</b> <u>S. Carneado Moreno, E. Díaz Riera, J.F. López-Sánchez, A. Sahuquillo.</u> <b>OR-16 Development and validation of LC-MS-based alternative methodologies to GC-MS for the simultaneous determination of triterpenic acids and dialcohols in virgin olive oil.</b> <u>Lucía Olmo-García, Aadil Bajoub, Romina P. Monasterio, Alberto Fernández-Gutiérrez, Alegría Carrasco-Pancorbo.</u> <b>OR-17 Dispositivos electrónicos como elementos de detección en ensayos genómicos de interés clínico.</b> <u>E.S. Yamanaka, L.A. Tortajada-Genaro, R. Puchades, A. Maquieira.</u> <b>OR-18 Detección de residuos de explosivos mediante espectrometría de masas con ionización de descarga de barrera dieléctrica.</b> <u>J. Robles-Molina, F.J. Lara-Ortega, D. Moreno-González, B. Gilbert-López, A. Schütz, S. Brandt, J. Franzke, J.F. García-Reyes, A. Molina-Díaz.</u> <b>OR-19 Real time colourimetric glucose determination in whole blood combining <math>\mu</math>TAD and smartphone.</b> <u>Miguel M. Erenas, Belén Carrillo, Isabel M. Pérez de Vargas Sansalvador, Kevin Cantrell, Sara González-Chocano, Ignacio de Orbe-Payá, Luis Fermín Capitán-Vallvey.</u>
13:25-15:30	<b>Sesión de posters – Comida</b>

15:30-16:05	<p><b>Conferencia invitada:</b>  <b>Multiplexed and Quantitative Bioanalysis using Surface Enhanced Raman Spectroscopy (SERS)</b>  <b>Karen Faulds</b>; <i>University of Strathclyde, Glasgow, UK.</i>  Modera: <i>Santiago Maspoch</i></p>
16:05-17:05	<p><b>Sesión comunicaciones orales:</b>  Modera: <i>M<sup>a</sup> Celia García Álvarez-Coque y Juan Fco. García-Reyes</i></p> <p><b>OR-20 Cancer cell targeting and specific delivery of silver nanoparticles by protein functionalized mesoporous silica-based nanocarriers.</b>  <i>Sandra Montalvo-Quirós, María Vallet-Regí, Blanca González, Jose L. Luque-García.</i></p> <p><b>OR-21 Modelado de propiedades de disolventes supramoleculares para procesos de extracción analítica.</b>  <i>J.A. Salatti-Dorado, D. García-Gómez, S. Rubio.</i></p> <p><b>OR-22 Bioconjugated metal nanoclusters: signal amplification and multiplexing capabilities for bioimaging of specific proteins in biological tissue sections by LA-ICP-MS.</b>  <i>María Cruz-Alonso, Eva Valencia-Agudo, Lydia Álvarez, Héctor González-Iglesias, Beatriz Fernández, Rosario Pereiro.</i></p> <p><b>OR-23 Rare earth elements to identify archaeological strata in the Cocina cave (Alicante, Spain).</b>  <i>Gianni Gallelo, Mirco Ramacciotti, Agustin Pastor, Oreto García Puchol, Agustin Diez, Sarah B. McClure, Joaquim Juan Cabanilles.</i></p>
17:05-17:30	<b>Café/Posters</b>
17:30-18:30	<p><b>Presentación / Discusión Posters</b>  Modera: <i>Pilar Viñas y Soledad Cárdenas</i></p>
18:30-19:15	<p><b>Entrega de premios/Ceremonia de clausura</b>  <b>Miguel Valcárcel, Pilar Campins (Vicerrectora Investigación UV) y Elena Domínguez</b></p>
21:00-24:00	<b>Cena del Congreso</b>

## JORNADA DE ESPECIACIÓN

**Comité Científico (Grupo de Especiación de la SEQA):**

- Tamara GARCÍA BARRERA (Presidenta, Universidad de Huelva)
- M<sup>a</sup> Carmen BARCIELA ALONSO (Secretaria, Universidad de Santiago de Compostela)
- Àngels SAHUQUILLO ESTRUGO (Vicepresidenta, Universidad de Barcelona)
- Jorge RUÍZ ENCINAR (Vocal, Universidad de Oviedo)
- Yolanda MADRID ALBARRÁN (Vocal, Universidad de Madrid)
- Francisco LABORDA GARCÍA (Vocal, Universidad de Zaragoza)

**Secretaría:**

- M<sup>a</sup> Luisa CERVERA SANZ (Universitat de València)

**MARTES 5 DE SEPTIEMBRE 2017**

11:00-11:30	<b>Recogida de documentación / Colocación de posters</b> Facultat de Filologia, Traducció i Comunicació. Avda. Blasco Ibáñez, núm. 32, 46010 – Valencia
11:30-11:45	<b><u>Presentación de la Jornada.</u></b> <i>Tamara García Barrera (Presidenta del Grupo de Especiación de la SEQA)</i> <i>Soledad Muniategui (Vicepresidenta SEQA)</i>
11:45-12:30	<b>Arsenic speciation in food: occurrence, analytical methods, risk assessment, risk management and toxicological update</b> <i>Tanja Schwerdtle; European Food Safety Authority (EFSA). Risk Assessment, University of Potsdam. Germany.</i>
12:30-13:00	<b>La problemática del análisis de especiación arsénico en alimentos.</b> <i>Josep Calderón; Laboratorio de la Agencia de Salud Pública de Barcelona (ASPB).</i>
13:00-14:00	<b><u>Round table:</u></b> <b>The role of speciation in food safety. Routine methods and regulations.</b> <i>Invitados: Tanja Schwerdtle, Josep Calderón</i>
14:00-15:30	<b>Comida</b>



15:30-17:00	<p><b>Sesión de comunicaciones orales.</b></p> <p><b>OR-ESP-01 Especiación de arsénico en fresas enriquecidas mediante HPLC-HG-AFS.</b>  <i>D. Sánchez-Rodas, I. Giráldez, F. Martínez, P. Palencia, A. González de la Torres</i></p> <p><b>OR-ESP-02 Especiación de selenio en muestras de pez espada y salmón, mediante HPLC-ICP-MS. Influencia del proceso de cocinado.</b>  <i>David Vicente-Zurdo, Beatriz Gómez-Gómez, María Teresa Pérez-Corona y Yolanda Madrid</i></p> <p><b>OR-ESP-03 Arsenic speciation in edible vegetables from northern Chile.</b>  <i>C. Elishian, S. Stegen, F. Queirolo, B. Torrejón-Vera, J.F. López-Sánchez, A. Sahuquillo</i></p> <p><b>OR-ESP-04 Simultaneous determination and speciation analysis of arsenic and chromium in iron supplements used for iron-deficiency anemia treatment by HPLC-ICP-MS.</b>  <i>U. Araujo-Barbosa, E. Peña-Vazquez, M.C. Barciela-Alonso, S.L. Costa-Ferreira, A.M. Pinto dos Santos, P. Bermejo-Barrera</i></p> <p><b>OR-ESP-05 Antimony speciation in spirits stored in pet containers.</b>  <i>S.Carneado, E. Klontzas, G.E. Froudakis, S.A. Pergantis, A. Sahuquillo, J.F. López-Sánchez</i></p> <p><b>OR-ESP-06 A universal solution to standardize absolute quantification of biomolecules using HPLC with ICP-MS detection.</b>  <i>Francisco Calderón Celis, Alfredo Sanz-Medel, Jorge Ruiz Encinar.</i></p> <p><b>OR-ESP-07 Cuantificación absoluta de proteínas mediante ICP-MS sin necesidad de patrones específicos: aplicación a la caracterización cuantitativa de venenos de serpiente.</b>  <i>L. Cid Barrio, F. Calderón Celis, S. Díez Fernández, J. J. Calvete, A. Sanz Medel, J. Ruiz Encinar.</i></p> <p><b>OR-ESP-08 Detección, caracterización y cuantificación de nanopartículas de óxido de titanio en muestras complejas mediante AF4-ICPMS.</b>  <i>David Ojeda, Vanesa Taboada-López, Eduardo Bolea, F. Laborda, Antonio Moreda, Juan Ramón Castillo, Pilar Bermejo.</i></p> <p><b>OR-ESP-09 Combinación de la extracción en punto de nube con TXRF y SP-ICPMS para la determinación de nanopartículas de plata en muestras acuosas.</b>  <i>L. Torrent, F. Laborda, M. Iglesias, E. Marguá, M. Hidalgo.</i></p>
17:00-18:00	<b>Sesión de posters y café</b>
18:00-18:15	<b>Entrega del Premio de la RSC a la mejor comunicación científica (Voucher para libros de 100€ de la RSC + diploma acreditativo)</b>
18:15-19:00	<b>Asamblea del Grupo de Especiación de la SEQA</b>

## **CONFERENCIAS**



## **Innovations in Mass Spectrometry Platform Technologies for Epithelial Ovarian Cancer Research**

**David Muddiman,**

W.M. Keck FTMS Laboratory for Human Health Research, Department of Chemistry, 603 Cox Hall, 2620 Yarbrough Dr. North Carolina State University, Raleigh, NC 27695-8204, USA

Mass spectrometry offers the most robust platform to discover and characterize new diagnostic, prognostic, and therapeutic biomarkers for ovarian cancer across all molecular classes. Moreover, a systems biology approach will allow the underlying biology of ovarian cancer to be understood. This presentation will discuss the challenges specific to the study of epithelial ovarian cancer (EOC) in humans and how these challenges have directed our thinking, in terms of the development of model organisms and mass spectrometry-based bioanalytical strategies. First, to augment the human model, we developed the domestic hen model of spontaneous EOC, which allowed us to longitudinally sample the rapid onset and progression of the disease in a controlled environment. Second, we developed bioanalytical tools to characterize structurally challenging analytes that are critical to a systems-level analysis. To increase the electrospray response of *N*-linked glycans, perform stable-isotope relative quantification, and semi-automated data analysis, we synthesized novel hydrophobic tagging reagents (INLIGHT™). Furthermore, we developed a novel ionization technique for tissue imaging of lipids and metabolites. This unique model organism has and continues to provide new insights into the biology of ovarian cancer; combined with other –OMICS data obtained through these novel bioanalytical approaches, we will understand the origin of ovarian cancer and ultimately translate that knowledge to humans.

## GCxGC-TOF-MS – A Powerful Technique for Environmental Studies

Lourdes Ramos

Department of Instrumental Analysis and Environmental Chemistry, IQOG-CSIC, Juan de la Cierva 3; 28006 Madrid, Spain

The chromatographic resolution offered by monodimensional gas chromatography (GC) has been demonstrated to be insufficient for the complete resolution of many complex environmental mixtures. In some cases, GC hyphenation with an appropriate mass spectrometric technique can contribute to solve the problem. However, in the case of trace components, appropriate and reliable identification and quantification is only achieved when both the sample preparation and the instrumental analytical conditions have been specifically developed and optimized for a certain type of sample and group of substances. This traditional *targeted* approach has been successfully used for several decades in many application studies, and it is the analytical strategy adopted in routine control programs at present. However, this approach has a significant drawback as it always will miss compounds, which were not selected at the start of the analyses. Therefore, in general, all unknowns or other untargeted substances even in high concentrations or with severe toxic potential will be missed.

At present, most environmental monitoring programs are based on the Stockholm Convention on persistent organic pollutants (POPs), which focuses on 26 chemicals or groups of chemicals. In contrast, there are approximately 100,000 industrial chemicals or chemicals of commerce used currently [1] and environmental samples contain hundreds of non-targeted compounds. Some of these untargeted compounds have recently been identified as novel bioaccumulative halogenated natural products, new isomers or metabolites of known contaminants [2], but also as non-previously described pollutants [3]. On the other hand, several screening computational studies have pointed out that, on the basis of their physico-chemical properties, many chemicals in use fulfill the Stockholm Convention criteria for POPs-like compounds. Among these bioaccumulative, persistent and long-range transport-capable chemicals, 98% are halogenated, and two-thirds are chlorinated, brominated and mixed halogenated compounds [1]. In other words, the number of potential POP-like compounds containing Cl and/or Br in their structure and reaching the environment is probably larger than the (rather limited) set of compounds monitored under the Stockholm Convention regulation.

This presentation discusses the potential of *non-targeted* comprehensive two-dimensional gas chromatography coupled to time-of-flight mass spectrometry (GCxGC-ToF MS) to assess the organohalogen burden of complex environmental samples. The benefits derived from the enhanced separation and identification power provided by this multidimensional technique will be illustrated using tuna muscle samples subjected to a rather generic sample preparation procedure as case study. The potential of classifications and script tools for automatic data filtering on the basis of specific structural characteristics will be discussed. Examples of legacy and non-legacy POP-like families, and novel chemicals detected in these samples will be provided.

**Acknowledgements.** Author thanks MINECO (AGL2016-80475-R; AEI/FEDER, UE), Fundación Ramón Areces, and FEDER program for funding.

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**Programming flow in capillary microfluidics: from concepts to assays****Emmanuel Delamarche**

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Diagnostics are ubiquitous in healthcare because they support prevention, diagnosis and treatment of diseases. Specifically, point-of-care diagnostics are particularly attractive for identifying diseases near patients, quickly, and in many settings and scenarios. One of our contribution to the field of microfluidics is the development of capillary-driven microfluidic chips for highly miniaturized immunoassays. In this presentation, I will review how to program capillary flow and encode specific functions to form microfluidic elements that can easily be assembled into self-powered devices for immunoassays. An important part of our research here deals with the integration of reagents and receptors to chips. Reagents such as detection antibodies must be carefully integrated for efficient release with well-controlled concentration profiles. Concerning the integration of receptors, we have developed a chip fabrication method using mild steps, which do not compromise biological reagents. Further, recent work suggests that capture antibodies can be integrated to microfluidic chips by self-assembling functionalized microbeads into a flow path. I will also present how small peripherals can augment the functionality of microfluidic chips that have integrated electrodes for example for monitoring flow with sub-nanoliter precision and for providing connectivity to smartphones. This can be done using a low-cost chip peripheral and tracking flow using near-real time capacitance measurements of a liquid front gradually wetting a pair of electrodes. Finally, counterfeiting of point-of-care diagnostics is an issue, with sometimes dramatic consequences. Using capillary phenomena, we devised a method for producing in the chip a complex signal with a “time domain” for authentication of devices. All together, capillary-driven elements can bring extremely high control for manipulating sub-microliter volumes of samples and picogram quantities of reagents and may therefore extend the performances of microfluidic devices for point-of-care diagnostics to a next level of precision.

## Nano-Liquid Chromatography: Main Features and Potentiality in Separation Science

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The introduction of miniaturization in liquid chromatography took place about three decades ago, as documented by the work of Novotny and Knox's groups. Since that time the research has focused great attention on development of theory, instrumentation (pumping, detectors systems), columns and stationary phases (SP) etc. In nano-liquid chromatography (nano-LC) capillaries with narrow internal diameter (10-100  $\mu\text{m}$  I.D.) containing selected SPs are currently employed, while in capillary liquid chromatography (CLC) 100-320  $\mu\text{m}$  I.D. columns are currently used. Mobile phases are pumped at relative low flow-rates (nL/min and mL/min, respectively). The low flow-rate is particularly helpful in i) reducing the chromatographic dilution, ii) increasing the mass sensitivity, iii) reducing costs (solvents and waste) and easier coupling with mass spectrometry (MS) [1]. In the last decade some companies proposed new instrumentation dedicated to nano-LC, mainly applied in the proteomic field. In general these apparatuses offered the possibility to carry out a fast pre-concentration step into a short column of analyzed tryptic digest proteins and then analytical separation utilizing nano-flow. At the same time nano-LC was also studied and applied in other fields such as pharmaceutical, forensic, environment, food, agrochemical etc. Although the good results achieved it is worth mentioning some drawbacks must be considered when deciding to use this miniaturized technique. First of all the extracolumn band broadening have to be minimized using, e.g., appropriate pumping system, detector at high frequency and low volume cell, tube connections and injection valve of low volumes (nL). In addition capillary columns containing SPs offering high efficiency and high selectivity are advised for successful sample analysis. To solve this last problem columns packed with particles with lower diameter (sub-2  $\mu\text{m}$ ) or with core-shell material have been proposed and successfully applied.

In this communication, a general overview about the features of nano-LC, considering all the above mentioned remarks, will be proposed. Several examples to resolve problems coming by the use of miniaturized instrumentation will be discussed. In order to document the potentiality of this technique, some applications, reported in literature, in the field of pharmaceutical, food, agrochemical will also be presented.

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## La Química Analítica frente a los retos de la globalización

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Se presentan una serie de reflexiones sobre el papel de la Química Analítica en un contexto globalizado. Se expondrán los retos que representa moverse en un nuevo entorno, muy permeable, en el que además se vislumbra un cambio de época. Se comentará el impacto y el significado que ello puede significar para la Química Analítica y para sus actores, desde diferentes ámbitos: formativo, académico, puramente científico y tecnológico.

La Química Analítica, sin duda, tiene por delante un periodo de gran protagonismo. En cualquier dirección que se mire existen importantes interrogantes a los que dar solución. Algunos ejemplos los encontramos en agroalimentación: autenticación de materias primas y productos procesados, nuevos productos, etc. En el ámbito de la energía también se vislumbran grandes cambios derivados de la “descarbonización de la economía”. No digamos del cambio climático, de la movilidad de la población (aumento de infecciones, “fiebre del viajero” o aparición de alergias).

Otro ámbito de gran interés para la Química Analítica es el de Salud y Bienestar. Así, en el estudio de enfermedades como el cáncer existen muchos ensayos basados en secuenciación génica, que es la técnica más usada y quizás la mejor, pero otras alternativas más sencillas e *in situ* o que prescindan de la etapa de amplificación, tienen gran porvenir. Otra revolución, ya en marcha, será desarrollar ensayos de diagnóstico *in vitro* sustitutivos de los clásicos *in vivo*. El envejecimiento de la población es un campo de trabajo sustancial en las sociedades más ricas, pero de crecimiento rápido en todo el mundo. La farmacogenética es otra área incipiente, donde la Química Analítica puede hacer grandes aportaciones, tanto en metodología como en interpretación de datos (quimiometría). El seguimiento y control de poblaciones (*point of need*, asistencia telemática, etc.) basado en tecnologías de telecomunicación es un campo novedoso que presenta desafíos muy importantes. Seguridad y contra-terrorismo también son áreas muy sensibles y con gran demanda de soluciones más prácticas que las actuales, aún poco efectivas. El transporte de mercancías también necesita de mejores soluciones, especialmente en aduanas, puertos y aeropuertos.

La industria está incorporando multitud de sensores, la mayoría físicos. Introducir sensores químicos con capacidad de analizar muestras mínimamente tratadas, es un tema complicado pero apasionante, y un gran reto para la Química Analítica.

A lo largo de la exposición se irán presentando, desde la apreciación personal del autor, ejemplos concretos, considerando los campos más interesantes y los enfoques más prometedores relacionados con el nuevo horizonte en el que la Química Analítica será un gran protagonista.



## **Multiplexed and Quantitative Bioanalysis using Surface Enhanced Raman Spectroscopy (SERS)**

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Surface enhanced Raman scattering (SERS) is an analytical technique with several advantages over competitive techniques in terms of improved sensitivity and multiplexing. We have made great progress in the development of SERS as a quantitative analytical method, in particular for the detection of DNA. However, the lack of quantitative data relating to real examples has prevented more widespread adoption of the technique. Detection of specific DNA sequences is central to modern molecular biology and also to molecular diagnostics where identification of a particular disease is based on nucleic acid identification. Many methods exist and fluorescence spectroscopy dominates the detection technologies employed with different assay formats. Another advantage of SERS over existing detection techniques is that of the ability to multiplex which is limited when using techniques such as fluorescence. We have clearly demonstrated the ability to identify the presence of a mixture of 6 analytes in solution using data analysis techniques.

Here we demonstrate the development of new molecular diagnostic assays based upon SERS which have been used successfully for the detection of DNA sequences from bacterial pathogens associated with meningitis using modified SERS active probes. The probes have been designed to give a specific SERS response resulting in discernible differences in the SERS which can be correlated to a specific DNA hybridisation event. The simultaneous detection and quantitation of 3 pathogens within a multiplex sample will be demonstrated for the first time.

We have also recently demonstrated the detection of pathogenic bacteria in food using functionalised nanoparticles combined with magnetic separation and SERS (Figure 1). Metallic nanoparticles have been functionalised with bio-recognition molecules (antibodies and lectins) which are specific for a bacterial strain and a Raman reporter to enable the SERS detection. A different Raman reporter was used for each strain of bacteria; therefore a SERS signal was only obtained when the SERS active nanoparticle binds specifically to its bacterial target. The aim is to develop a multiplexing assay where three bacterial strains can be simultaneously identified in a sample matrix.

## **PRESENTACIONES TÉCNICAS**



**ANALYSIS OF MYCOTOXINS  
IN FOOD MATRICES**

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Mycotoxins are compounds produced by fungi that grow on crops ranging from grains to fruits, vegetables, nuts, and spices. Mycotoxins can be harmful to humans and livestock through consumption of contaminated crops; therefore, mycotoxin levels are monitored in foods to minimize the risk of ingestion<sup>1</sup>. Regulatory agencies around the globe set Maximum Residue Limits (MRLs), which range from <10 to >500 ppb to ensure harmful levels of mycotoxins do not enter the food supply. It is important to detect and accurately quantify mycotoxin contents at low levels across various food matrices, as each matrix composition poses different detection challenges. This study demonstrates the accurate and sensitive quantification of up to 12 regulated mycotoxin compounds in three commonly regulated foods using the Agilent Ultivo Triple Quadrupole LC/MS.

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**NUEVO ICP-MS “NEXION 2000”: CUALQUIER MATRIZ - CUALQUIER INTERFERENCIA -  
CUALQUIER TAMAÑO DE PARTÍCULA**

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Introducción al nuevo equipo de ICP-MS presentado por PerkinElmer en 2017, en donde se detallarán las novedades que incorpora, entre otras la nueva fuente de RF, el nuevo sistema de introducción de muestra, nuevas posibilidades de corrección de interferencias y los nuevos accesorios disponibles.

[1] PerkinElmer Inc., 2017, Brochure Reference: 012730A\_ESP\_01 (12 pages)

## **COMUNICACIONES ORALES**



**SHEATHLESS CAPILLARY ELECTROPHORESIS-MASS SPECTROMETRY IN THE  
METABOLIC PROFILING OF BIOMASS-RESTRICTED SAMPLES. METABOLOMIC  
ANALYSIS OF A MOUSE MODEL OF POLYCYSTIC KIDNEY DISEASE**

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Metabolomics or metabolic profiling is the unbiased analysis of the end products and intermediates of the metabolism occurring in an organism. Heterogeneity of the physicochemical properties of the metabolites implies the use of multiple analytical techniques. Among these techniques, Capillary Electrophoresis coupled to Mass Spectrometry (CE-MS) and, sheathless CE (CESI) platform in particular [1] has a strong track-record as a tool to profile polar and ionizable compounds, especially for volume or biomass restricted samples. The CESI platform is widely used in metabolomics research, but to the best of our knowledge it has never been used in metabolomic analysis of tissue samples. Thus, the main objective of this work has been to show the feasibility of the CESI platform in the metabolomic analysis of biomass-restricted samples, namely 20 µm-thick kidney sections of mice. In order to demonstrate the potential of the sheathless CE-MS in metabolic analysis it was applied to the metabolic profiling of a mouse model of Polycystic Kidney Disease (PKD). PKD is a disease based on the development of cysts in kidneys [2]. More particularly, autosomal PKD has a prevalence from 1 per 500 to 1 per 1000 people globally, requiring in most cases dialysis or renal transplantation by age 55 [3]. The use of mouse models is a very informative tool which provides information which would not be obtainable by human observational studies.

The sheathless CE-MS platform was proven to obtain rich information regarding metabolic profiling despite the use of such biomass-restricted samples, being necessary volumes as low as 2.5 µL in the injection vial. The method based on a background electrolyte composed of 10 % (v/v) acetic acid (pH 2.3) and an injection volume of roughly 9 nL enabled to determine different compound classes in tissue sections, including amino acids, betaines, purines and nucleosides, among others.

Both supervised and non-supervised multivariate analysis enabled to differentiate between the different PKD stages despite the restriction in the amount of the analyzed samples. A set of metabolites changing over experimental groups could be annotated. Creatine and creatinine levels decreased as disease progressed. Glutamine also decreased with progression of PKD as observed in previously literature [4]. This demonstrates the potential of sheathless CE-MS in metabolomic analysis of biomass-restricted samples.

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**“ESTRATEGIAS ANALÍTICAS BASADAS EN ESPECTROMETRÍA DE MASAS EN LA BÚSQUEDA DE NUEVOS BIOMARCADORES DEL CÁNCER DE MAMA: LA MMP-11**

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Las metaloproteinasas de matriz (MMPs) son una familia de endopeptidasas dependientes de zinc, cuya principal función es la degradación del tejido conectivo del estroma y los componentes de la membrana basal que son elementos clave en el crecimiento tumoral, invasión, metástasis y angiogénesis. En particular, la expresión de MMP11 por las células mononucleares inflamatorias del estroma intratumoral se asocia con la progresión del tumor y el mal pronóstico del cáncer de mama. En consecuencia, la MMP-11 involucrada en estos procesos puede ser un nuevo biomarcador pronóstico en este tipo de cáncer (1).

Surge la necesidad de métodos analíticos sensibles y selectivos que permitan la determinación los niveles de MMP11 en fluidos biológicos y/o su distribución en tejido de mama de personas sanas y con patología de cáncer de mama. La espectrometría de masas de plasma de acoplamiento inductivo ICP-MS resulta de gran utilidad para la determinación elemental, sin embargo no proporciona información de las biomolécula asociada al metal. La combinación de técnicas inmunoensayo con ICP permite la determinación específica y sensible de (metalo)proteínas (2).

Asimismo, las técnicas analíticas de bioimagen basadas en LA-MS son una herramienta de gran utilidad en biología y medicina en estudios de sistemas biológicos para proporcionar información de biomoléculas y metales con resolución lateral a escala micrométrica. En particular, la técnica de ablación laser acoplada a la espectrometría de masas de plasma de acoplamiento inductivo (LA-ICP-MS) ha sido desarrollada para el mapeo elemental de elementos traza esenciales (oligoelementos) en tejidos biológicos. Alternativamente, la espectrometría de masas de desorción / ionización asistida por matriz (MALDI-MS) para la obtención de imágenes y de la distribución de los biomarcadores de proteínas implicados en el cáncer de mama con alta sensibilidad y alta resolución local (3).

En este trabajo se puso a punto una metodología para la determinación por ICP-MS en combinación con el inmunoensayo para la determinación los niveles de MMP11 en muestras de suero y cultivos celulares de personas sanas y con patología mamaria. Asimismo, se ha puesto a punto una metodología para la estudiar la distribución elemental de los elementos esenciales (Fe, Cu, Zn y Ca) por LA-ICP-MS y el mapeo de MMP11 por LA-MALDI en los tejidos de cáncer de mama.

Los resultados por LA-ICP-MS muestran distribución heterogénea de Ca, Fe, Cu y Zn en tejido de cáncer de mama, siendo significativamente mayores en el área tumoral en comparación con los niveles en el área no tumoral. Asimismo, los resultados por LA-MALDI muestran niveles más altos de MMP-11 en el tejido tumoral en comparación con el sano, indicando un metabolismo alterado y señalando así a MMP-11 como un posible biomarcador candidato para diagnóstico y pronóstico del cáncer de mama. Se discutirá la sinergia de las técnicas de espectrometría de masas de imagen elemental y molecular.

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# **ELEMENT-TAGGED IMMUNOASSAYS WITH ICP-MS DETECTION FOR QUANTIFICATION OF SOME IRON-REGULATORY PROTEINS IN BREAST CANCER CELLS: ON THE SEARCH FOR CANCER BIOMARKERS**

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Breast cancer is a major cause of morbidity and mortality in women all over the world. Therefore, the search for biomarkers for early detection, prognosis and predicting drug responses in breast cancer is a growing field of research. In this regard, recent experiments have linked a deregulation of iron homeostasis with breast cancer progression, aggressiveness and recurrence [1].

Intracellular iron homeostasis is tightly regulated by a number of proteins including transferrin and ferritin, which are involved in cellular Fe uptake and Fe storage, respectively. Therefore, the relationship between these iron-regulatory proteins and cancer has become increasingly evident. In fact, elevated expression of transferrin and ferritin has been associated to the progression of breast cancer cells towards a more malignant phenotype [2]; however, the underlying molecular mechanisms for the cancer-linked ferritin and transferrin alterations remain largely unknown and often with conflicting conclusions.

In order to investigate the roles of these proteins in breast carcinogenesis, highly sensitive analytical methods are required to perform their quantification in cancer cells with high precision and accuracy. For this purpose, here we present a mass spectrometry-based analytical strategy (inductively coupled plasma-mass spectrometry, ICP-MS) combined with antibody labelling in a sandwich assay format for ferritin [3,4]; and transferrin determination. The developed methodologies involves two monoclonal antibodies against the targeted protein, one of them biotinylated and the other one labeled with a ruthenium chelate in the case of ferritin quantification or with iodine in the case of transferrin quantification. The complex formed in solution between the protein (ferritin or transferrin) and the two antibodies is then captured using streptavidin-coated magnetic microparticles and directly introduced into ICP-MS for Ru or I monitoring. In addition, here we also present the possibility of using the combination of immunoassays with ICP-MS measurements to address the Fe content in the corresponding isolated ferritin [4].

The developed element-tagged immunoassays with ICP-MS detection were applied to the analysis of two breast cancer cells of different malignancy. The information obtained, taken altogether with results on the total cytosolic Fe concentration, the non-ultrafiltrable Fe concentration and the Fe-speciation experiments (by Size Exclusion Chromatography (SEC)-ICP-MS) will be used to discuss Fe-metallomics in the context of breast cancer.

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## SCREENING OF NEW PSYCHOACTIVE SUBSTANCES IN URINE SAMPLES BY HIGH RESOLUTION MASS SPECTROMETRY

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In the last decade, the widespread use of new psychoactive substances (NPS) raised global concern due to the high number of different substances not regulated available on the market and their high potential toxicity for human health. These substances are synthesized adding or changing a functional group of an already controlled drug molecule, so they have similar psychoactive properties of traditional drugs of abuse [1]. To obtain reliable information about the consumption of NPS the main tools are epidemiological surveys and the analysis of biological samples, mainly urine. However, the continuous synthesis and marketing of new NPS make the identification of these substances an analytical challenge.

The aim of this work was to screen the presence of selected NPS in urine samples collected during music festivals in Europe and evaluate their consumption. A list of 190 NPS was created according to the frequency of detection reported by the European Early Warning System [2], and included synthetic cannabinoids and cathinones, phenethylamines, synthetic opioids, tryptamines, piperidines, aminorex derivatives, natural NPS, benzodiazepines and ketamine analogues. The qualitative analysis of NPS was carried out using high-resolution mass spectrometry (HRMS, Q-Orbitrap analyzer). A first screening was performed followed by a non-target analysis based on data-independent acquisition mode. The proposed target NPS were confirmed by the measurement of the corresponding standard when available.

Among the substances investigated, only the synthetic cathinone 4-chloro-alpha-pyrrolidinopropiophenone (4-Cl- $\alpha$ -PPP) was identified and confirmed in the analyzed samples by acquiring the corresponding analytical standard. As far as we know, this is the first time this synthetic cathinone was identified in urine sample from music festivals.

**Acknowledgements:** This work was supported by the project NPS- Euronet (HOME/2014/JDRU/AG/DRUG/7086). N. Salgueiro thanks Xunta de Galicia-GAIN for her postdoctoral grant (Modalidade A, 2016).

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# LC–QTOF MS/MS ANALYSIS OF CHANGES IN THE COMPOSITION OF THE POLAR FRACTION OF PERSIAN LIME (*CITRUS LATIFOLIA*) DURING FRUIT GROWTH

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A large number of studies has demonstrated that citrus fruits are rich in bioactive compounds. However, only few of them have evaluated changes in their chemical composition during ripening<sup>[1-2]</sup>. The traditional ripening indicators –titratable acidity, soluble solids or color– are strongly related to fruit taste<sup>[3]</sup>. Therefore, the evaluation of the metabolites composition during ripening can be helpful to obtain fruits with better nutraceutical properties. The presented study was aimed at establishing the differences in Persian lime (*Citrus latifolia*) sampled from weeks 1 to 14 of maturation using liquid chromatography–tandem mass spectrometry (LC–QTOF MS/MS). The samples (96) were collected by random coordinates in the experimental field, immediately frozen by liquid nitrogen and lyophilized prior metabolite extraction. Persian lime extracts were obtained by ultrasound assisted extraction (53% of ethanol in water; 5 min; 70% amplitude; 0.9 s/s) using the method previously developed by the authors<sup>[4]</sup>. Each extract was analyzed by LC–QTOF MS/MS in both positive and negative ionization modes.

After processing the LC–QTOF MS/MS data, the global data set (96x423) was used to study the differences among samples by both ANOVA and Tukey test ( $p \leq 0.01$ ), which showed that the number of significantly different molecular entities increased when differences in growth times among samples were longer. To complete the untargeted analysis, a PCA was applied, which showed a clear differentiation among samples from different growth weeks. Based on the MS/MS information, the tentative identification of 72 metabolites (28 flavonoids, 5 phenolic acids, 3 coumarins, 14 amino acids, 8 carboxylic acids, 6 sugars, and 8 other compounds pertaining to different classes) was achieved. The changes in concentration of all single identified metabolites during fruit growth were evaluated. Regarding phenols, phenolic acids were generally more concentrated during the early stages and then decreased significantly after 7 weeks, flavanones and flavones had maximum concentration between weeks 9 to 12, and flavanols and coumarins exhibited their maximum concentration during the first weeks, decreasing as ripening progressed. Phenylalanine, a precursor in the phenols pathway, exhibited a sinusoidal behavior along Persian lime growth, coinciding its lowest concentration with the maximum concentration of phenolic acids and flavonoids. Concerning sugars, sucrose, fructose and glucose showed maximum concentrations between weeks 7 and 12, which decreased significantly at the end of the ripening period. On the other hand, the concentration of carboxylic acids gradually increased during fruit growth, with a maximum between 9 and 14 weeks, except for the ascorbic acid, which reached maximum concentration at week 12 and decreased at the final of the ripening process. Finally, essential amino acids did not show significant changes during fruit development, except for phenylalanine. The results here presented open the possibility of improving the quality of this fruit, and performing new evaluations of the role of citrus fruits as a source of nutraceuticals for a comprehensive exploitation of this crop.

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# NOVEL AND VERSATILE MICROEXTRACTION APPROACHES BASED ON DISPERSION OF MAGNETIC MATERIALS COATING STIR BARS

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Nowadays, sample preparation is one of the most studied areas in Analytical Chemistry, especially in trace analysis where it is usually necessary to perform preconcentration of analytes and a clean-up step to eliminate potentially interfering compounds. For this purpose, microextraction techniques play a critical role since they allow both enrichment and separation of the analytes from the sample matrix in a single step. Among the vast array of microextraction techniques currently developed, magnetic-based approaches have gained popularity due to the magnetic properties of the extraction phases that allow their facile and fast retrieval by means of an external magnetic field.

With the aim to contribute to the development and improvement of microextraction techniques, our group has developed a hybrid microextraction approach termed stir bar sorptive-dispersive microextraction (SBSDME) [1-3]. In this novel technique, a magnetic sorbent material (e.g.  $\text{CoFe}_2\text{O}_4$ @oleic acid magnetic nanoparticles or  $\text{CoFe}_2\text{O}_4$ @ $\text{SiO}_2$ -nylon 6 magnetic composite) is added to a sample vial containing a neodymium bar-shaped magnet, in such a way that it is attracted by the magnet. At low stirring rate, the material remains in the stir bar as in stir bar sorptive extraction (SBSE), whereas at higher stirring rates the material is released and dispersed into the donor solution as in dispersive solid-phase extraction (DSPE), thus increasing the surface area of the extraction phase. When the stirring process is ceased, the magnetic material returns to the magnetic bar without requiring an additional external magnetic field like for example in DSPE. The coated stir bar containing the extracted compounds is easily removed from the sample solution and the analytes can be either desorbed in an appropriate solvent for further injection into a liquid chromatographic (LC) instrument [1,3], or directly thermally desorbed into a gas chromatographic (GC) system [2].

Recently, we have introduced a novel microextraction approach in which the magnetic sorbent was replaced by a magnetic ionic liquid (MIL). In this technique, termed stir bar dispersive liquid microextraction (SBDLME) [4], the MIL is dispersed at high stirring rate similarly to dispersive liquid-liquid microextraction (DLLME), in such a way merging the advantages of SBSE and DLLME.

The analytical advantages of these novel approaches have been demonstrated in the determination of different target analytes, such as UV filters, polycyclic aromatic hydrocarbons and endocrine disruptors in different types of matrices like waters, coastal sand and urine.

## Acknowledgements

Authors acknowledge the financial support of the Spanish Ministry of Economy and Competitiveness (Project CTQ2015-70301R).

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**EXTRACCION MEDIANTE ELECTROMEMBRANA INTEGRADA EN UN DISPOSITIVO MICROFLUÍDICO PARA LA DETERMINACIÓN DE FLUOROQUINOLONAS EN AGUAS MEIOAMBIENTALES**

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En este trabajo, se presenta la optimización y la puesta a punto de un dispositivo microfluídico que integra la microextracción mediante electromembrana ( $\mu$ -EME) para la determinación de fluoroquinolonas y su posterior análisis mediante HPLC-UV. Para el estudio se estudiaron las cinco fluoroquinolonas de uso más frecuente: marbofloxacina (MRB), norfloxacin (NPF), danofloxacin (DNF), ciprofloxacin (CPF) y flumequina (FLM).

El dispositivo microfluídico consiste en dos micro-canales (aceptor y donador) separados por una membrana plana de polipropileno. En cada una de las fases (donadora y aceptora) se coloca un electrodo de platino. De esta manera, los analitos se extraen desde la fase donadora hasta la fase aceptora a través de la membrana líquida soportada gracias al campo eléctrico creado a ambos lados de la membrana cuando se aplica una diferencia de potencial entre los electrodos. El pH de la fase donadora y aceptora fue 11 y 10, respectivamente.

Este dispositivo microfluídico permite obtener una elevada eficiencia de extracción en tiempos de extracción de 5 minutos, un excelente “*clean-up*” y reduce significativamente el volumen de muestra (< 10  $\mu$ L) empleando flujos de 1  $\mu$ L / min.

**Agradecimientos:** BP B-Marie Curie (0025) (the 7th Framework Programme European Commission) y el Ministerio de Economía, industria y competitividad- contrato Juan de la Cierva Incorporación.

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## NANOMETROLOGÍA ANALÍTICA: HACIA LA RESOLUCIÓN DE PROBLEMAS COMPLEJOS

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El desarrollo de la Nanotecnología y la Nanociencia está dando lugar a nuevos retos y oportunidades para la Química Analítica. Las nanopartículas no consisten sólo en entidades químicas, con una composición determinada, sino también entidades físicas, con tamaño y forma definidas, junto con una serie de características superficiales que les añaden una complejidad adicional.

Frente a la Nanometrología, que se define como la ciencia de la medida aplicada a la nanoescala (<100 nm), y la Nanometrología Química, que cubre los aspectos químicos, básicamente de composición, nosotros hemos acuñado el término Nanometrología Analítica como la Ciencia Analítica aplicada a la nanoescala, básicamente orientada a la detección, caracterización y cuantificación de nanomateriales en muestras de complejidad diversa.

El uso de nanomateriales en distintos tipos de escenarios (alimentación, salud, medio ambiente, estudios de (eco)toxicidad...) está generando una serie de problemas analíticos que implican el desarrollo de técnicas, métodos y enfoques novedosos que permitan obtener la información demandada desde distintos sectores científicos, tecnológicos y sociales [1]. En el caso de los nanomateriales inorgánicos, hemos apostado por una plataforma multimetodológica [2] basada en el uso de: (i) la espectrometría de masas con fuente de ionización ICP (ICPMS), utilizada en modo convencional y de detección de partículas individuales (single particle-ICPMS [3]), así como acoplada a técnicas de separación en función del tamaño (fraccionamiento en flujo mediante campos de flujo asimétrico [4], cromatografía hidrodinámica); (ii) técnicas electroanalíticas [5] (voltametría de partículas inmovilizadas, coulombimetría de colisión de partículas, sensores); (iii) técnicas de microscopía electrónica (transmisión y barrido de campo extendido). Por otra parte se han desarrollado métodos de especiación por HPLC-ICP-MS (6), MIPS-HPLC-ESI-MS(7), y métodos de CV-ICP-OES asociados a técnicas de extracción con MIPS (8). Este conjunto de técnicas se complementa con distintos tipos de tratamientos previos de las muestras (digestiones enzimáticas, centrifugación, ultrafiltración, extracción punto de nube, etc...).

El objetivo de esta comunicación es presentar las posibilidades de una plataforma multimetodológica desarrollada al amparo de un proyecto coordinado entre grupos analíticos de la U de Zaragoza y la U de Santiago de I+D+I del Programa Estatal de I+D+I orientada a los retos de la sociedad, evaluando sus prestaciones y limitaciones a través de una serie de problemas analíticos seleccionados que implican obtener información sobre nanomateriales, tanto sintéticos como naturales, en muestras de distinta complejidad.

Este trabajo ha sido subvencionado por el Ministerio de Economía y Competitividad y el Fondo Europeo de Desarrollo Regional, proyecto CTQ2015-68094-C2-1-R (Univ Zaragoza) y CTQ2015-68094-C2-2-R (Univ Santiago) (MINECO/FEDER).

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# DETECTION OF B-TYPE NATRIURETIC PEPTIDE (BNP) AT SCREEN PRINTED CARBON ELECTRODES MODIFIED WITH GOLD NANOPARTICLES GRAFTED THROUGH ARYL DIAZONIUM SALT CHEMISTRY

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A key step in the proper functioning of an electrochemical biosensor is the surface architecture connecting the biological sample to the transducer. The type of materials involved, its geometry and size strongly affect the response of the biosensor. Furthermore, gold nanoparticles (AuNPs) have a strong capacity for biomolecules adsorption without losing of biological activity and facilitate the electron transfer between redox biomaterial and the electrode surface. Thus, immobilization of AuNPs on bulk electrodes using covalently attached organic layers containing thiol groups and amino groups on their distal end offers a simple, fast, and versatile approach for preparing nanoparticle electrode arrays with low background capacitance.

On the other hand, since the signs and symptoms of heart failure (HF) are not very specific and the availability of echocardiography is limited, a very interesting alternative approach to diagnosis HF is to determine accurately the blood concentration of natriuretic peptides (NPs). Between them, the B-type natriuretic peptide (BNP) is a 32-amino acid polypeptide secreted by the ventricles of the heart in response to excessive stretching of heart muscle cells. Interestingly, it has been found that BNP concentration in serum is elevated in HF patients compared to controls, establishing a cut-off value of 400 pg mL<sup>-1</sup>.

With the purpose to develop a competitive electrochemical strategy for the accurate determination of clinically relevant levels of BNP in serum samples, in this work, we have prepared a sensitive electrochemical immunosensor for this polypeptide determination based on the immobilization of capture antibodies onto gold nanoparticles grafted through aryl diazonium salt chemistry onto screen-printed carbon electrodes (SPCEs) by means of a sandwich type immunoassay involving a peroxidase-labeled detector antibody. This novel nanostructured platform resulted in an ordered layer of gold nanoparticles adsorbed onto 4-aminothiophenol which combines the advantages of the high conductivity and the stability of immobilized biomolecules. To monitor the extension of the affinity reactions the amperometric transduction at disposable screen-printed carbon electrodes was performed at -200 mV (vs the Ag pseudo-reference electrode) upon addition of hydroquinone (HQ) as electron transfer mediator and H<sub>2</sub>O<sub>2</sub> as the enzyme substrate.

After optimizing all the experimental variables affecting the operation and response of this immunosensing approach its analytical performance was characterized and their practical applicability successfully demonstrated by analysis of real human serum samples.

The promising analytical performance exhibited by this new amperometric immunosensor of simple operation, disposable SPCE format and possibility to use pocket-size electrochemical transducers makes it very attractive alternative to commonly used ELISAs for the development of automated POC systems for on-site determination of this powerful biomarker associated with HF.

The financial support of projects: Retos Colaboración RTC-2015-4184-1 (cofinanced by the Ministry of Economy and Competitiveness and FEDER "una manera de hacer Europa"), CTQ2015-70023-R and CTQ2015-64402-C2-1-R (MINECO Research Projects) and S2013/MT-3029 (NANOAVANSENS Program from the Comunidad de Madrid) are gratefully acknowledged.



**DIRECT ELECTROCHEMICAL TESTS ON URINE AS NON-INVASIVE STRATEGY FOR DETECTING UROTHELIAL CARCINOMA**

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About 9% of all incidental malignant disorders in the urinary tract are seen as urothelial carcinoma, commonly known as bladder cancer. Although there is disposal of several citological, molecular and genetic markers, the diagnosis of bladder cancer is usually achieved by means of biopsy obtained during cystoscopy, a time-consuming, invasive technique which remain as the gold standard [1]. In order to provide non-invasive methods for diagnosis, the analytical performance of electrochemical measurements in spontaneously voided urine samples at glassy carbon and gold electrodes were tested. The method was tested by means of a clinical trial with a control group of healthy volunteers (15) and a set of 21 patients diagnosed of bladder cancer at pTa, pT1 and pT2 stages. The patients were randomly selected from population performing routine bladder cancer screening and from selected patients prior to surgery. According to the requirements of our ethics committee, all participants provided signed informed consent. Voltammetric patterns were dominated by the signals between +0.4 and +1.2 V vs. Ag/AgCl attributable to the oxidation of uric acid and metabolites of serotonin, melatonin and tryptophan, among others which appear to be involved in urothelial carcinomas [2], consistently with data for artificial urine enriched in the different model compounds of such metabolites. The used methodology, inspired in the voltammetry of microparticles philosophy [3] becomes sensitive to changes in the relative concentrations of the metabolites which are reflected in variations in the relative height of the respective voltammetric peaks. Using shape-depending parameters, two-dimensional peak current ratios provide tendency lines discriminating between healthy urine and samples from patients diagnosed of bladder cancer. Combining the matrix of peak current (normalized to uric acid)/applied potential data for gold and glassy carbon working electrodes, screening of pTa-, pT1- and pT2-diagnosed patients can also be attained, a result of particular interest in the context of clinical practice.

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## EVALUATION OF MULTIVARIATE CALIBRATION OF ISOBARIC COMPOUNDS IN A FIA-MS/MS CONTEXT

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The use of non-separative analytical strategies is a very attractive alternative due to the reduction in time analysis, especially when a large number of samples has to be processed. Separation of the individual compounds of a sample is not performed and a signal profile of the sample formed by all the components integrating it is obtained.

Within this context, multivariate calibration has been used to get quantitative information from profile signals. Several steps are followed. First, it is necessary to model the relationship between dependent (y) and independent variables (x) using what is called training set. Later on, the model must be validated by predictions of samples from whose concentrations are known. Finally, the calibration model is used for quantification of new samples<sup>1</sup>.

This approach has been broadly used with different non-separative analytical instrumental configurations, such as electrochemical sensors<sup>2</sup> or direct coupling of a headspace sampler with a mass spectrometer (HS-MS)<sup>3,4</sup>, among others.

Here, an evaluation of the analytical possibilities of multivariate calibration for individual quantification of isobaric compounds in a flow injection tandem mass spectrometry (FIA-MS/MS) context is presented.

FIA-MS/MS, as well as HS-MS, could be considered as an open sensor array in which each m/z ratio or multireaction monitoring (MRM) transition acts as a 'sensor' that detects any ion fragment or analyte breakdown with that value. Thus, the number of sensors is variable, readily modifiable and, in most cases, high.

As instrumental configuration, a LC-MS/MS system consisted of a 1200 series HPLC system with a binary pump, a membrane degasser, an autosampler, a six-port valve, a diode-array detector and a 6410 LC/MS triple quadrupole (QqQ) mass spectrometer, all from Agilent Technologies (Waldbronn, Germany). The triple quadrupole mass spectrometer was equipped with an electrospray (ESI) source.

As target analytes, as mentioned above, isobaric compounds were considered, such as leucine and isoleucine, among others. These compounds are difficult to quantify individually due to co-elution problems or the presence of similar fragmentation patterns. Different MS acquisition modes were evaluated in order to obtain the best profile data and partial least squares (PLS) regression was proposed.

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**SAMPLE INTRODUCTION SYSTEMS INVOLVING LOW SAMPLE CONSUMPTION FOR MULTI-ELEMENT DETERMINATIONS IN SERUM BY ICP-MS**

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Alzheimer's disease (AD) is a type of dementia that causes loss of cognitive abilities and even produces behavioural disorders. The main symptom is loss of memory, although with the evolution of the disease, other abilities are lost. Some studies have suggested trace elements imbalance in patients suffering AD, and trace elements are therefore being included in blood analysis. However, the assessment of trace and ultra-trace elements in clinical samples is difficult because of the complexity of the sample, the low target concentrations, and also the low volumes/amount of samples typically available for analysis. Cerebrospinal fluid is a recommended sample for neurodegenerative diagnosis. However, serum is preferred since sampling is easier and less painful for the patient.

The aim of the current research has been the development and validation of news methodologies to achieve a simultaneous, fast, and accurate determination of trace and ultra-trace elements in serum samples. Inductively coupled plasma – mass spectrometry (ICP-MS) offers excellent sensitivity for multi-element determinations, but requires a certain volume of sample, which is higher as the number of targets increases. Therefore, we have optimized two different sampling modes for multi-element measurement by ICP-MS, such as laser ablation (LA), and direct introduction of low volumes of sample by using the SeaFast autosampler system. Samples analysed by LA-ICP-MS have required a sample pre-treatment based on Dried Blood Spot (DBS) technique, for which only 20 µL of serum sample is used (the serum drop is spotted onto a filter paper). A 1:10 dilution with 1.0 % (v/v) nitric acid has been needed when using the SeaFast autosampler system (for a total volume of 2.0 mL, only 200 µL of serum is required).

Both sample introduction methods have been evaluated/compared for the direct determination of trace elements in serum at low µg L<sup>-1</sup> concentrations. Methods validation has been performed by analysing several certified reference materials, and both methods have been applied to several serum samples from healthy and patient adults.

**Acknowledgement**

The authors belong to the Galician Competitive Research Group 6RC2014/2016. This program is co-funded by FEDER (UE).

## MEASURING HOLD-UP VOLUME AND PHASE RATIOS IN HILIC

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Since distribution constants between mobile and stationary phases are directly related to retention factors, a proper and accurate measurement of this parameter is fundamental in retention modeling and in the determination of thermodynamic and kinetic parameters related to chromatographic retention.

There are several methods reported in the literature for the determination of hold-up volumes: pycnometry, homologous series, unretained neutral markers and inorganic salts. Because of its ease of use, UV absorbing analytes, non-polar neutral molecules for normal-phase (tetrachloromethane, benzene, toluene...) and inorganic salts for reversed-phase ( $\text{NaNO}_3$ ,  $\text{KBr}$ ...) were proposed as suitable markers. However, due to the complexity of separation mechanisms in hydrophilic interaction chromatography (HILIC) the selection of a suitable hold-up time marker is challenging. It is assumed that the main retention mechanism is based on the partition of solutes between the bulk mobile phase and a water enriched layer that is semi-immobilized on the stationary phase. Thus, non-polar hydrophobic substances appear to be suitable markers. McCalley and Neue [1] studied the retention of benzene and toluene in different silica columns using acetonitrile/water mobile phases, and they found that retention depended on the fraction of water in the eluent. Later studies performed in HILIC columns showed that water uptake depends not only on the particular functionality of the stationary phase (underivatized silica, amide, aminoalkyl, sulfoalkylbetaine, phosphorylcholine...) but also on the interactive layer between the silica support and the functionality (conventional silane chemistry, polymer grafting...) [2,3].

In the present work a new general method for measuring hold-up volume based on linear free energy relationships (LFER) [4] has been developed and applied to HILIC. The method is based on the measurement of the retention times ( $t_R$ ) of homologous series for which the hold-up time ( $t_0$ ) is related to the molar volume of the analyte ( $V$ ) through the following equation:

$$t_R = t_0 + r 10^{vV}$$

where  $t_0$ ,  $r$ , and  $v$  are fitted parameters from measured  $t_R$  and calculated molar volumes ( $V$ ).

Two different homologous series showing different specific solute-solvent interactions (except hydrogen-bonding acidity),  $n$ -alkyl benzenes and  $n$ -alkyl phenones, were studied, and acetonitrile and methanol were assayed as mobile phase organic modifiers. In contrast to reversed-phase mode, retention times decreased with the molecular volume of the homologous series, suggesting the partition of solutes between the bulk mobile phase and the water enriched layer as a key retention mechanism.

The combination of the new LFER method for hold-up time determination with the classical pycnometry for overall eluent volume measurement allows the calculation of the phase ratios ( $V_0/V_s$ ), and thus the determination of both the hold-up ( $V_0$ ) and the stationary phase ( $V_s$ ) volumes.

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## SIMULTANEOUS DETERMINATION OF ADDITIVES IN FOOD PRODUCTS BY DIGITAL IMAGE ANALYSIS

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A method based on digital image is described to quantify tartrazine and allura red colorants in food samples. HPLC is the habitual method of reference used for colorant separation and quantification, but it is expensive, time-consuming and it uses solvents, sometimes toxic. By a flatbed scanner, that can be found in most laboratories, images of mixtures of colorants can be taken in microtitration plates. Only 400  $\mu\text{L}$  of sample are necessary and up to 92 samples can be measured together in the same image acquisition. A simple-to-obtain color fingerprint is obtained by converting the original *RGB* image into other color spaces and individual PLS models are built for each colorant. In this study, errors of 9.47%, 12.8% and 13.6% for tartrazine and 5.52%, 7.22% and 8.83% for allura red have been obtained for calibration, cross-validation and external validation respectively. Results for repeatability and reproducibility are under 12%. These results are slightly worse but comparable to the ones obtained by HPLC. The applicability of both methodologies to real food samples has proven to give the same result provided that large interferences are not present and color additives are not at very low concentrations. Considering the colorant content found in most samples this should not be a problem though. Values of LODs of 1.8  $\text{mg L}^{-1}$  and 0.6  $\text{mg L}^{-1}$  for tartrazine and allura red respectively have been obtained by image analysis.

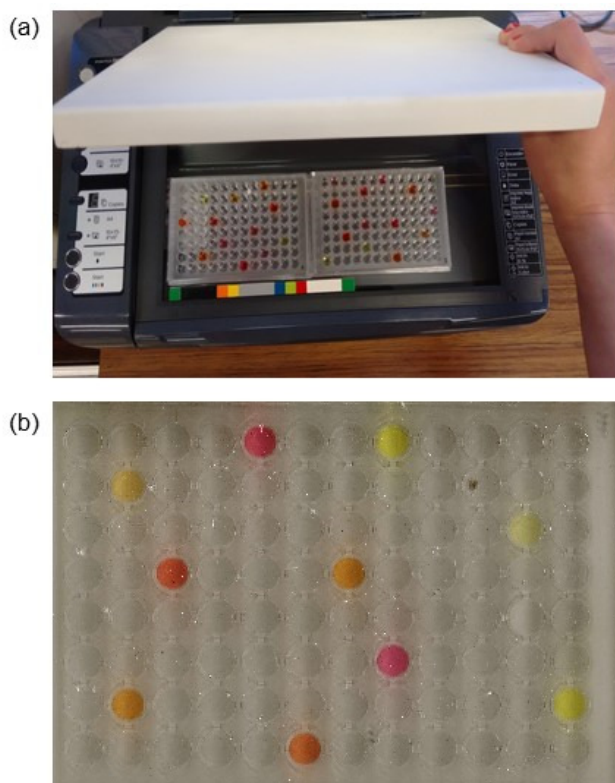


Figure 1. (a) Image showing the used system to acquire the digital images. Two microtitration plates have been placed in the scanner window together with a color standard for illumination control. Several wells are full with samples with different colorant mixture content. (b) Obtained scanned image of one microtitration plate.

**ANTIMONY MIGRATION STUDIES IN FRUIT JUICES STORED IN  
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Total antimony contents from 100 to 400 mg kg<sup>-1</sup> have been reported in PET containers, irrespective of the commercial brand tested. In the major of cases, they are destined to the storage of mineral water. Such studies concluded that the presence of Sb in the bottled water was due to its migration from PET, as water obtained from a fresh source or stored in plastic of other types did not suffer from Sb contamination. Much less information has been reported concerning total antimony contents in other beverages bottled in PET, which have more complex matrices including considerable amounts of salts or organic acids, such as soft drinks, red wine and fruit juices.

In this work, the determination of total antimony and the redox species Sb(V) and Sb(III) were assessed in seven PET-bottled juices from different fruit matrices. Reproducible and reliable results were obtained with the analysis by ICP-MS with a single sample pre-treatment (centrifugation and dilution of the supernatant) providing results between 0.17 – 0.94 µg L<sup>-1</sup> for total Sb contents.

The influence of temperature and storage time up to 60 days on Sb migration from PET bottles into peach and pineapple juices was also studied. Sb migration was assayed by ICP-MS for total determination and LC-ICP-MS for speciation analysis. The study showed that storage at 4°C and 20°C did not present significant migration over time, whereas a significant increase on Sb concentration was observed at 40°C and 60°C. Pineapple juice showed more pronounced migration than peach juice. The maximum limit established in drinking waters by the European Union (5 µg Sb L<sup>-1</sup>) was not exceeded in any of the juice samples.

Regarding speciation analysis, Sb(III) was the predominant redox species present in the juices which migrated from the PET bottles throughout the experiments. MS spectrometry analyses demonstrated that this form is present in samples as a 1:2 Sb(III)-citrate complex, which is converted into 1:1 Sb(III)-EDTA in the presence of the mobile phase during the analysis by LC-ICP-MS. The concentration levels followed the same tendency as described for total Sb analysis. Small amounts of non-complexed Sb(V) were also observed near the limit of detection, together with some unidentifiable signals of very low intensity. Storage at 4°C and 20°C did not result in a significant evolution of species concentrations. Sb(III) concentrations in the samples stored at 4°C and 20°C were 0.13 ± 0.02 and 0.27 ± 0.04 µg Sb L<sup>-1</sup> for peach and pineapple respectively. Storage at 40°C and 60°C presented a slight increase in Sb(III) concentration up to day 30.

**DEVELOPMENT AND VALIDATION OF LC-MS-BASED ALTERNATIVE METHODOLOGIES TO GC-MS FOR THE SIMULTANEOUS DETERMINATION OF TRITERPENIC ACIDS AND DIALCOHOLS IN VIRGIN OLIVE OIL**

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Pentacyclic triterpenes are minor, but very relevant compounds found in virgin olive oil (VOO). GC with FID or MS as detectors is considered the gold standard tool to determine these analytes. Few examples can be also found in literature regarding their determination in VOO by LC-MS, but generally without giving quantitative data of each analyte in terms of their own standard (but referring them to another analyte) and not determining both triterpenic acids and dialcohols within a single run.

A rapid and reliable LC-MS method for determining both types of compounds from VOO within the same run (after ultrasound assisted extraction) has been developed, giving a tangible alternative to GC (FID/MS) methodologies. The analytical parameters of the proposed method were exhaustively checked, establishing limits of detection (from 1 to 95 µg/l) and quantification, precision (RSD values for inter-day repeatability were found between 4.2 and 7.3% considering area values), trueness (within the range 92.7 and 100.5%) and evaluating possible matrix effect (which was no significant). The method was applied to the analysis of six triterpenic compounds in 11 monovarietal VOOs and the results compared with the quantitative GC-MS data. Moreover, the direct injection (after a simple dilution) of the samples into the LC-MS system was also tested, in an attempt to proffer an even simpler sample treatment.



## DISPOSITIVOS ELECTRÓNICOS COMO ELEMENTOS DE DETECCIÓN EN ENSAYOS GENÓMICOS DE INTERÉS CLÍNICO

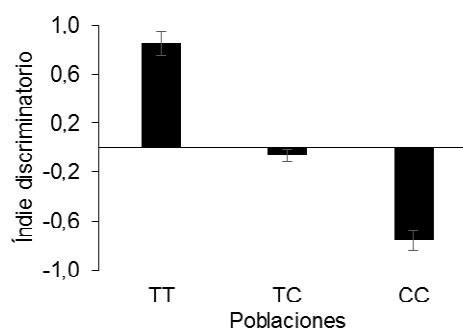
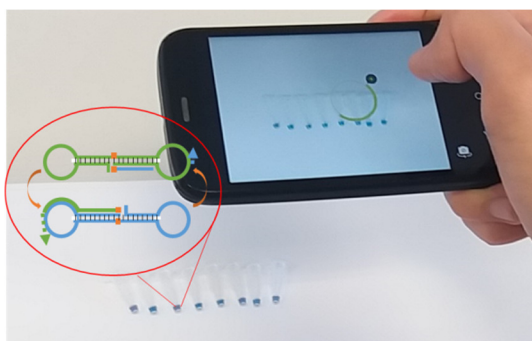
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Las tecnologías moleculares para la detección de biomarcadores genéticos están permitiendo avances considerables en el diagnóstico y el pronóstico de las enfermedades [1]. En particular, están abriendo nuevas oportunidades en el tratamiento personalizado, lo que permite aumentar la eficacia de los fármacos y reducir sus efectos negativos. La implementación de este nuevo paradigma de la medicina, exige afrontar importantes retos analíticos. Se necesitan metodologías de ensayo con alta sensibilidad y selectividad, y que sean competitivos para la rutina clínica en términos de facilidad de uso, rapidez y costo. En este sentido, se han desarrollado diferentes plataformas tipo *point-of-care* que generalmente incluyen un dispositivo de detección portátil y un ensayo de ADN simple, sensible y preciso [2-3].

En esta comunicación, se presentan los resultados de la integración de metodologías génicas y tecnologías de detección innovadoras que sean alternativas a técnicas como la secuenciación o PCR a tiempo real. La investigación ha abordado el modo de lograr una respuesta analítica selectiva en función de los alelos presentes a partir de una muestra de fluido biológico del paciente. Para ello, se ha estudiado la amplificación isoterma como estrategia que evita el uso de termocicladores y permite la miniaturización del ensayo [4]. Se han optimizado las condiciones de la amplificación isotérmica mediada por bucle (LAMP) para aumentar el número de copias de la región diana, con rendimiento superior una PCR convencional [5]. Se han comparado dos aproximaciones: hibridación alelo-específica de los productos y amplificación utilizando cebadores específicos de alelos. Se ha estudiado un marcaje colorimétrico, basado en el revelado inmunoenzimático, y el sistema pirofosfato/magnesio/azul del hidroxinaftol, respectivamente. La detección óptica se ha logrado mediante integración con dispositivos electrónicos de consumo: microscopio USB digital, escáner de documentos, *smartphone* y unidad de DVD.



Como prueba de concepto, se ha desarrollado un método que actúa como herramienta farmacogenética para el tratamiento de fármacos anti-depresivos. Se trata de un ensayo diagnóstico sensible, reproducible y rápido que logra la identificación del genotipo de un polimorfismo (SNP rs1954787) en un gen implicado en la neurotransmisión (*GRIK4*). A partir de muestras de saliva de los pacientes, ha sido posible la asignación al subgrupo correspondiente, siendo los resultados coincidentes con los obtenidos mediante los métodos de referencia.

**Agradecimientos.** Proyecto MINECO CTQ2013-45875-R, FEDER GVA Prometeo II 2014/040

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## DETECCIÓN DE RESIDUOS DE EXPLOSIVOS MEDIANTE ESPECTROMETRÍA DE MASAS CON IONIZACIÓN DE DESCARGA DE BARRERA DIELECTRICA

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La detección de explosivos es un reto importante para la seguridad pública a nivel mundial. Compuestos nitroaromáticos como el trinitrotolueno (TNT) y las nitroaminas como la hexahidro-1,3,5-trinitro-1,3,5-triazina (RDX) se encuentran entre los explosivos más comunes. Éstos, a veces, vienen formulados con otras especies químicas como el nitrobenzeno (NB), dinitrobenzeno (DNB) y nitrotolueno (NT). La presión de vapor de explosivos como el RDX o el TNT suele ser muy baja. La detección de estas sustancias requiere de métodos altamente selectivos, sensibles y la tecnología empleada debe ser capaz de trabajar con compuestos no volátiles y térmicamente inestables, así como ofrecer tiempo de respuesta real y potencial de portabilidad. En este sentido, la fuente de ionización por descarga de barrera dieléctrica (DBDI) es de gran interés, ya que es capaz de trabajar como fuente de desorción/ionización para espectrometría de masas en ambiente [1] y también como una técnica de ionización para el acoplamiento de cromatografía de líquidos y espectrometría de masas (LC-MS) [2].

En este trabajo se ha estudiado la detección de explosivos y otros compuestos relacionados por infusión directa en DBDI y con el acoplamiento LC-DBDI-TOFMS. Para este propósito se escogieron un total de 15 compuestos: 2-nitrotolueno, 3-nitrotolueno, 4-nitrotolueno, 2,3-dinitrotolueno, 2,4-dinitrotolueno, 2,6-dinitrotolueno, 3,4-dinitrotolueno, 1,2-dinitrobenzeno, 1,4-dinitrobenzeno, 1,3,5-trinitrobenzeno, 2-amino-4,6-dinitrotolueno, 4-amino-2,6-dinitrotolueno, RDX, octogen (HMX) y TNT. El cromatógrafo de líquidos (Agilent 1290 Infinity HPLC) estaba acoplado a un espectrómetro de masas con analizador de tiempo de vuelo (Agilent 6220 TOF) trabajando en modo de ionización negativo. Para la separación cromatográfica se utilizó una columna analítica Zorbax RRHD eclipse plus C18 de dimensiones 3.0 mm i.d. x100 mm, 1.8 µm. La fuente DBDI se implementó a través de la modificación de una fuente comercial de ionización química a presión atmosférica (APCI Agilent), de manera que el flujo procedente del LC es nebulizado y evaporado de la misma manera que en APCI; sin embargo, la aguja de la corona es reemplazada por el microplasma generado por descarga de barrera dieléctrica. Los parámetros DBDI fueron 2.5 kV y 100 ml/min de flujo de He. Debido a que se estudiaron compuestos con distinta volatilidad, siendo alguno de ellos termolábiles, se estudiaron 4 combinaciones de temperaturas de nebulizador-vaporizador; 200-225 °C, 250-275 °C, 300-325 °C y 325-375 °C. También se realizó un estudio del efecto de la composición de las fases móviles (A: H<sub>2</sub>O-MeOH 90-10, y B: MeOH-H<sub>2</sub>O 50-50) sobre la generación de distintos iones para cada compuesto seleccionado, en presencia o no de modificador químico (acetato de amonio). El resto de parámetros experimentales permaneció constante. Los resultados mostraron que las temperaturas de la fuente y composición de las fases móviles, afectaban a la intensidad y naturaleza de los iones que se formaban y, como se esperaba, RDX y HMX al ser termolábiles daban mayor señal cuando las temperaturas de la fuente eran menores.

### Agradecimientos

Los autores agradecen el apoyo de la Junta de Andalucía" (Ref. FQM-2242) y del Ministerio de Economía y Competitividad (CTQ-2012-34297).

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## REAL TIME COLOURIMETRIC GLUCOSE DETERMINATION IN WHOLE BLOOD COMBINING $\mu$ TAD AND SMARTPHONE

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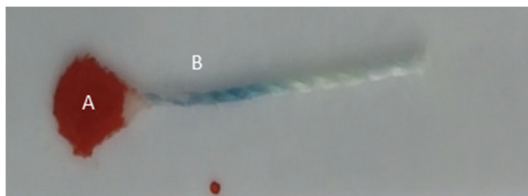
In recent years, thanks to the high processing power of smartphones together with their ability to digitalize objects using an integrated image detector, it has become possible to develop different sensors that can be carried to the place where they are needed and obtain the analytical parameter and analyte concentration[1]. The image detector digitalizes the sensor and an application calculates the analytical parameter, usually a colour coordinate of the sensor, once the developed reaction has finished.

Currently, some of the most commonly used sensors with smartphones are microfluidic, because of the low sample volume needed to make them work and the possibility of integrating different analytical operations that allow the user to directly apply the sample to the sensor without any pre-treatment.

In this study, we have developed a thread-based microfluidic analytical device ( $\mu$ TAD)[2] for the determination of glucose directly on a whole blood sample without any prior pre-treatment of the sample. The analytical device is based on the colourimetric method used for the determination of glucose in plasma using glucose oxidase and horseradish peroxidase, with the appearance of a blue colour that is related to the glucose concentration.

In order to use the whole blood directly, the  $\mu$ TAD (see Figure 1) separates the blood cells from the plasma thanks to a separation membrane included in the sensor design, after which the plasma is buffered to 7.4 pH and the blue colour depending on the glucose concentration develops in a few seconds. The colour change of the  $\mu$ TAD is recorded by a smartphone in a video file that is analysed in real time, such that when the R/B from the RGB colour space reaches its maximum, it saves the value and interpolates it in the calibration function. The glucose concentration of the sample analysed then appears on the screen.

With the  $\mu$ TAD, a smartphone and the application, the user can obtain a glucose concentration from a sample in only 20 seconds using the colourimetric method.



**Figure 1.**  $\mu$ TAD design for glucose determination in whole blood. A) Blood cell separation membrane; B) Colour change produced by plasma reaching the detection area.

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Acknowledgements: This work was funded by Spanish Ministry of Economy and Finance projects CTQ2013-44545-R and CTQ2016-78754-C2-1-R.

## CANCER CELL TARGETING AND SPECIFIC DELIVERY OF SILVER NANOPARTICLES BY PROTEIN FUNCTIONALIZED MESOPOROUS SILICA-BASED NANOCARRIERS

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Nanotechnology keeps gaining interest in the biomedical field due to the wide variety of nanomaterials-based applications in diagnostics and therapeutics. Specially, mesoporous silica nanoparticles (MSNs) are considered promising nanocarriers for controlled and stimulus-responsive drug delivery [1]. One of the most attractive properties of this material is their silanol rich surface, which can be easily functionalized with organic structures such as proteins or DNA. In cancer disease, the overexpression of endogenous transferrin receptor (TfR) is a potential marker of tumoral cell population [2-3], which makes it a good candidate target for the design of cell specific drug delivery nanosystems [4]. It is well-known that silver nanoparticles (AgNPs) have an efficient and broad-spectrum antibacterial activity and, recently, they are also receiving considerable attention as potential anticancer therapeutic agents [5]. However, AgNPs tend to aggregate and its administration could also provoke cytotoxic effects on healthy cells [6]. Immobilization of AgNPs in a supporting matrix together with transferrin as a cancer cell targeting ligand, would avoid AgNPs aggregation while maintaining the possibility of its delivery in a cell specific manner.

For this reason, the aim of this study has been the design, synthesis and *in vitro* evaluation of a hybrid nanosystem consisting of metallic AgNPs well dispersed on MSNPs and externally functionalized with transferrin for cancer cell targeting.

We have synthesized fluorescent MSNs through a base-catalyzed sol-gel process in the presence of a structure directing agent. We have covalently attached transferrin (targeting ligand) or BSA (control) onto the external surface of MSNs, followed by incorporation of AgNPs by reduction of a silver ion precursor (AgNO<sub>3</sub>). We optimized this process using different amounts of silver ion precursor and reducing agents. We characterized the nanomaterials by elemental analyses, X-ray diffraction, N<sub>2</sub> adsorption, DLS and zeta-potential measurements, and scanning and transmission electron microscopy. Then, we evaluated the potential of the synthesized nanosystems *in vitro* by bioanalytical strategies (cell viability and flow cytometry assays) to estimate the efficiency of the targeting, the degree of cellular internalization and the potential anticancer effects of the delivered AgNPs in several cell lines: human keratinocytes (HaCaT), human epithelioid cervix carcinoma cells (HeLa) and human hepatocarcinoma cells (HepG2) [7].

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## MODELADO DE PROPIEDADES DE DISOLVENTES SUPRAMOLECULARES PARA PROCESOS DE EXTRACCIÓN ANALÍTICA

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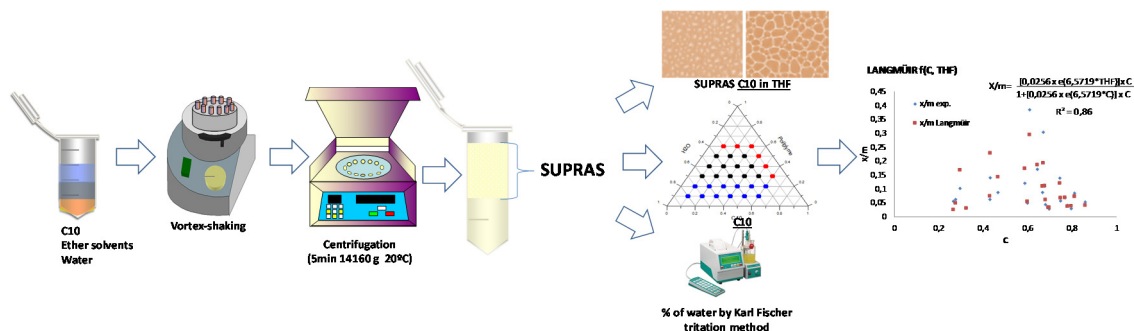
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Los disolventes supramoleculares (SUPRAS) pueden definirse como fases ricas en tensioactivo con organización a dos niveles, nano y microscópico. Se obtienen a partir de disoluciones acuosas o hidro-orgánicas de tensioactivo mediante procesos de autoensamblaje y coacervación. Su aplicación en procesos de extracción analítica es un área en expansión dada la elevada eficacia de extracción proporcionada por los SUPRAS para compuestos en un muy amplio intervalo de polaridad y peso molecular, y en muestras de diferente naturaleza y complejidad.

Una de las características más relevantes de los SUPRAS es la posibilidad de diseñar disolventes con propiedades programadas para que cumplan funciones específicas. Esta característica deriva de la reversibilidad/reorganización de las estructuras supramoleculares que lo integran en respuesta a modificaciones ambientales. Así, se han obtenido SUPRAS a partir de alcoholes alquílicos y ácidos carboxílicos en medio THF-agua que muestran propiedades de acceso restringido, lo que ha permitido la integración de la extracción y limpieza de la muestra en una única etapa.

Hasta la fecha, el diseño de SUPRAS se ha basado en ensayos de prueba y error y el estudio de sus propiedades en el ajuste de ecuaciones matemáticas a los resultados experimentales. Dado que el diseño y producción de disolventes con propiedades programadas aumenta notablemente la capacidad de mejorar la selectividad, rendimiento y costes de los procesos de extracción, sería de gran interés el desarrollo de modelos teóricos que permitan predecir las propiedades de los SUPRAS en función de las condiciones ambientales.

Se presenta en esta comunicación un primer modelo teórico, basado en una modificación del modelo clásico de adsorción de Langmuir, para SUPRAS sintetizados a partir de ácidos carboxílicos en medios hidro-orgánicos. Este modelo permite predecir, a partir de la composición inicial del medio hidro-orgánico, la composición acuosa de los agregados hexagonales inversos que constituyen el SUPRAS. Esto permite, por consiguiente, estimar el tamaño de la cavidad acuosa de los mismos, y, como consecuencia, evaluar su capacidad para la exclusión de compuestos en base a su tamaño molecular. Su aplicabilidad se ha comprobado para uno de los tensioactivos más utilizados (ácido decanoico) y para muy diversos disolventes orgánicos de naturaleza etérea (THF, dioxano, dioxolano y mono-, di-, tri-, tetra- y poli-glima). En todos los casos, el ajuste de los datos experimentales al modelo planteado fue muy satisfactorio ( $r > 0,9$ ). Cabe destacar que el desarrollo de este modelo teórico no solo permite predecir la composición del SUPRAS y las propiedades que de ella se derivan, sino que también establece las bases para un conocimiento más profundo de estos sistemas supramoleculares. Por ejemplo, el modelo planteado responde a la ecuación de Van't Hoff, lo que permite la obtención directa de parámetros termodinámicos como la entalpía y la entropía del proceso de autoensamblaje del SUPRAS.



**Agradecimientos:** Los autores agradecen el apoyo financiero del MINECO (Proyecto CTQ2014-53539-R) y FEDER. J.A.S.D. y D.G.G. agradecen igualmente sus contratos de postgrado (FPU13/03796) y postdoctoral (FJCI-2014-20052), respectivamente.

**BIOCONJUGATED METAL NANOCLUSTERS: SIGNAL AMPLIFICATION AND  
MULTIPLEXING CAPABILITIES FOR BIOIMAGING OF SPECIFIC PROTEINS IN  
BIOLOGICAL TISSUE SECTIONS BY LA-ICP-MS**

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Laser ablation (LA) coupled to inductively coupled plasma-mass spectrometry (ICP-MS) is currently regarded as a powerful inorganic tool for solids trace element and isotopic analyses. Among the most interesting analytical features of LA-ICP-MS are its sensitivity in the ng/g range and a spatial resolution in the  $\mu\text{m}$  range. In addition, the combination of immunoassays with LA-ICP-MS can facilitate protein bioimaging in biological tissues using proper metal-tagged immunoprobos.

Metal chelates coordinated with heteroatoms (typically lanthanide ions) have been reported for elemental tagging [1]. It was also described the use of polymeric tags containing metal ions, such as MaxPar<sup>TM</sup> [2]. These polymeric tags show signal amplification (up to 177 lanthanides per antibody) compared to metal chelates. However, these labels are composed of a polymeric non-metallic part, giving rise to a relatively high size, which could impede an efficient antibody-antigen interaction (or even restrict the permeability accessibility of the bioconjugate inside tissue cell). Also they show the risk of unspecific interactions with the tissue. In this context, the use of metal nanoclusters (NCs) as tags is a promising alternative: the ratio "number of metal atoms/size" in a NC is very high as compared to a polymeric tag because it does not contain carbon or other nonmetals, so higher amplification can be achieved with a smaller size (each NC can have about 500 metal atoms with typical diameters of 2-3 nm).

In this communication we will present immunohistochemical procedures based on AuNCs and AgNCs synthesized in our laboratory (using lipoic acid as stabilizing ligand) and bioconjugated with selected antibodies *via* carbodiimide crosslinking [3] for bioimaging of specific proteins in biological tissue sections by LA-ICP-MS (the use of a different metal for each protein allows for multiplexing of different proteins). As a proof of concept, bioimages of metallothionein 1/2 and metallothionein 3 in ocular and cancer tissue sections will be shown. Results demonstrated that under the selected operating conditions, unspecific interactions are avoided and highly resolved images can be obtained (the great amplification provided by the NC allows to use very small laser spot sizes). Conventional fluorescence immunohistochemistry was also performed to validate molecular images obtained with the proposed methodology using LA-ICP-MS.

Acknowledgements: Project ref. CTQ2016-79015-R (AIE/FEDER, UE).

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**RARE EARTH ELEMENTS TO IDENTIFY ARCHAEOLOGICAL STRATA IN THE  
COCINA CAVE (ALICANTE, SPAIN)**

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Rare earth elements (REE) have been employed in a variety of different scenarios in order to identify the natural or anthropogenic nature of archaeological soils [1,2] .

In this study, REE signatures were employed to better understand the layers formation in a cavity called Cocina cave, a large cavity of 300 m<sup>2</sup> located at Barranco de la Ventana, one of the ravines flowing southwards from La Canal valley, a little plateau located in the municipality of Dos Aguas (Valencia, Spain). Cocina cave is characterized of very homogenous sediment deposition where it is difficult to understand layers formation processes just employing the traditional archaeological methods and the standardized soil analyses. The archaeological sequence encompasses last hunter-gatherer Holocene occupations in the regional sequence (Mesolithic) followed by several levels attributed to the Neolithic, Bronze Age and historic occupations until the XX century, these last regarding the use of the cavity as a pen.

In order to understand the development history of the strata and the anthropogenic or natural formation of soils a total of fifty samples were taken across six different sections (A, B, C, D, E, F, G) and from each section the sampling was carried out at different depths through 1-2m deep sections. All samples were recovered from current pits excavated at the cavity corresponding with some profiles that encompass different strata including natural deposits and hunter and penning activities together with other possible uses not well defined from archaeological data. Several radiocarbon dates confirm the anthropogenic use of the cavity from the IX millennium cal BP to the contemporary times. Major, minor and trace elements including REE were determined employing XRF and ICP- MS. Results were then statistically processed and cross-referenced with archaeological data to aid interpretation.

The results show that REE provide interesting details regarding the strata development history, and therefore help archaeologists to better understand the occupation, use and abandonment phases of the cave.

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## **COMUNICACIONES FORMATO PÓSTER**





**HPLC-UV FINGERPRINTING FOR THE CLASSIFICATION AND AUTHENTICATION OF EXTRA VIRGIN OLIVE OILS BASED ON MULTIVARIATE CALIBRATION METHODS****N. Carranco<sup>1</sup>, M. Farrés-Cebrián<sup>1</sup>, J. Saurina<sup>1,2</sup>, O. Núñez<sup>1,2,3</sup>**<sup>1</sup> Department of Chemical Engineering and Analytical Chemistry, Faculty of Chemistry, University of Barcelona. Martí i Franquès, 1-11, 08028, Barcelona.<sup>2</sup> Research Institute in Food Safety, University of Barcelona, Recinte Torribera, Av. Prat de la Riba 171, Edifici de Recerca (Gaudí), 08921, Santa Coloma de Gramenet, Barcelona.<sup>3</sup> Serra Húnter Fellow, Generalitat de Catalunya, Spain.e-mail: [oscar.nunez@ub.edu](mailto:oscar.nunez@ub.edu)

Virgin olive oil (VOO) is a vegetable oil obtained by mechanical or direct pressing of the pulp of the olive fruit (*Olea europaea* L.) which is the primary fat source of the Mediterranean diet. The olives, after being crushed to form a pomace, are homogenized and pressed. This oil is not subjected to any other treatment except for washing, decantation, centrifugation or filtration. The oil produced from this first press is known as extra-virgin olive oil (EVOO), and today it is considered the highest quality olive oil, characterized by containing the highest levels of beneficial constituents [1]. Apart from the natural unrefined state, olive oils can also be consumed as a lower quality refined product that is made from virgin olive oils and called refined olive oils (ROO) or refined olive-pomaces. Olive oils have become a very important factor in the Mediterranean diet providing beneficial health effects on the cardiovascular system because of their antioxidant capacity, mainly due to the presence of phenolic compounds. However, lately, there is the suspicion among consumers and administrations that, in some cases, olive oils have been adulterated with oils obtained from more economic fruit/seeds such as sunflower, corn, or soy, as well as the fact that some VOOs or even ROOs have been labeled as EVOOs by some producers in order to increase profits. This is a relevant problem not only because of the financial issues involved in main olive oils producing countries such as Spain or Italy but also for a health concern and the impediment of the consumer protection. Moreover, recognition of some frauds is especially difficult, e.g., when the cheaper oil is added to olive oils at a level lower than a certain percentage. Therefore, the reduction of the current levels of fraud in olive oils demands the constant development of new analytical techniques for the detection of possible adulterations.

In this work, a simple, cheap and reliable high performance liquid chromatography method with ultraviolet detection (HPLC-UV) for the determination of polyphenolic profiles in the classification and authentication of olive oils was developed. A total of 138 olive oils were processed by liquid-liquid extraction using ethanol:water 70:30 v/v solution and defatted with hexane [2]. The extracts were then analyzed by reversed-phase chromatography using a Zorbax Eclipse XDB-C8 column under gradient elution with 0.1% formic acid aqueous solution and methanol as mobile phase. First, 72 vegetable oils including 47 EVOOs, 16 sunflower oils, 2 corn oils, 2 soy oils, and 5 vegetable oils produced from mixtures of seeds were analyzed. HPLC-UV chromatographic fingerprints recorded at different acquisition wavelengths were submitted to exploratory principal component analysis (PCA). Good classification and discrimination of EVOOs against other vegetable oils was achieved. Then, characterization and classification of EVOOs regarding the olive cultivar of origin was attempted. For that purpose, 66 monovarietal EVOOs (23 Arbequina, 19 Picual, 12 Hojiblanca and 12 Cornicabra) were analyzed and the obtained HPLC-UV data submitted to PCA. In this case, the selection of some specific chromatographic time-window segments was required to achieve a satisfactory discrimination of EVOOs regarding the olive cultivar of production.

Finally, partial least squares (PLS) regression was employed to determine the percentage of adulterations in EVOOs. For that purpose, an Arbequina EVOO was adulterated (from 2.5 to 85% adulteration levels) with a Picual EVOO, a ROO, and a sunflower oil. The results showed that even mixture samples containing low percentages (2.5%) of adulteration could be distinguished from genuine EVOO samples with overall quantitation and prediction errors below 2.88%.

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# LIQUID CHROMATOGRAPHY WITH ULTRAVIOLET AND AMPEROMETRIC (SCREEN-PRINTED ELECTRODES) DETECTION FOR THE DETERMINATION OF POLYPHENOLS IN THE CHARACTERIZATION AND CLASSIFICATION OF SPANISH PAPRIKAS

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In recent years, there has been an increase interest in diets rich in fruits and vegetables due to the presence of bioactive compounds, such as polyphenols and carotenoids, and their presumed role in the prevention of several degenerative diseases, such as cancer and cardiovascular diseases. Polyphenols are aromatic secondary metabolites ubiquitously spread through the plant kingdom comprising more than 8000 substances with highly diverse structures. These compounds are mainly formed by aromatic rings bonded to one or more hydroxyl groups and they are generally involved in the protection against ultraviolet radiation or aggression by pathogens, and characterized mainly by their antioxidant properties. Today polyphenols offer many benefits and they are even been used as functional foods [1].

Paprika, or chilli pepper, is a red powder seasoning with a characteristic flavour obtained from the drying and grinding of certain varieties of red peppers. The two most known varieties of paprika in Spain, and the only ones with product designation of origin (PDO), come from the region of La Vera in Cáceres (Extremadura) and from Murcia. There are three important varieties: sweet, bittersweet, and spicy paprika, and the Vera ones are characterized by their smoky aroma produced during the drying process by the smoke of oak woods. Polyphenols are among the most interesting bioactive compounds found in paprika, and their distribution may be attributed to their different red pepper varieties. Nowadays, the interest in developing analytical methodologies for the determination of polyphenols as well as to guarantee PDOs as an important and remarkable product quality factor is raising [2].

In this work, a simple, cheap and reliable high performance liquid chromatography (HPLC) method with ultraviolet (UV) and amperometric detection (using screen-printed electrodes, SPE) for the determination of polyphenolic profiles in the characterization and classification of PDO paprika samples was developed. Chromatographic separation on a Kinetex C18 reversed-phase (100 x 4.6 mm, 2.6 µm particle size) core-shell column was proposed under gradient elution based on 0.1% (v/v) formic acid aqueous solution and methanol for the determination of 17 polyphenolic compounds, allowing to obtain phenolic profiles in less than 20 min. For amperometric detection, several SPEs (carbon, carbon nanotubes, carbon nanofibers, graphene and high and low temperature gold) were employed, and the best results regarding polyphenolic detection and sensitivity were obtained with carbon nanofiber SPEs with an oxidation potential of 1.1 V. The developed methods showed good sensitivity, precision and trueness. Sample treatment was carried out by liquid-liquid extraction. Several extracting solutions (water, ethanol, acetone, methanol, and acetonitrile) at different ratios were evaluated for the Paprika sample extraction with the aim of achieving discriminating polyphenolic profiles. The best results were obtained when water:acetonitrile (20:80 v/v) solution was employed. A total of 96 paprika samples that differ between region (72 from La Vera and 24 from Murcia) and variety (spicy, sweet and bittersweet) were analysed. HPLC-UV and amperometric polyphenolic chromatographic fingerprints were then analyzed by principal component analysis (PCA) to gain information on the most significant compounds contributing to characterization and classification of PDO paprikas. PCA results showed a noticeable separation among paprika PDOs. Besides, a very good discrimination of sweet, bittersweet and spicy varieties for both PDO evaluated paprikas was also encountered.

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**FLAME ATOMIC ABSORPTION SPECTROMETRY FOR DIRECT ZnONPS ANALYSIS IN WATER AND FRUIT JUICE. EFFECT OF FOOD MATRIX IN ZnONPS STABILITY AND BIOACCESSIBILITY****B. Gómez-Gómez, M.T. Pérez-Corona and Y. Madrid**

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Nowadays nanotechnology has a great impact in many industrial and technological sectors. For example in the food industry, the development of more efficient food packaging materials is being considered through the use of nanoparticles [1]. In particular, ZnO nanoparticles (ZnONPs) are widely studied during the last years for their employment in food packaging due to their exceptional antimicrobial properties [2]. However, the application of nanoparticle in the food industry may lead with their migration into the comestible products causing potential toxicity through oral ingestion. Although there are several studies in the literature concerning the impact of ZnONPs in human health, most of them are related to their use in sun creams and cosmetic products [3]. In contrast, very few studies are focused on toxicity through their oral ingestion as well as their potential transformation through the gastrointestinal tract and additionally, the effect of food matrix on ZnONPs stability. Therefore, the development of easy, sensitivity, fast and affordable analytical methods to determine NPs in complex matrices are required.

This study describes the development of an analytical methodology based on the use of FAAS to directly quantify mass concentration of ZnONPs spiked in food matrices (water and fruit juice). In order to assess the influence of the matrix composition in ZnONPs stability, ZnONPs were first synthesized and subsequently characterized in different dispersion media: Milli-Q water, citric acid, ascorbic acid, orange juice and pineapple juice. Compared to aqueous dispersions, a decrease in size, along with a partial dissolution of ZnONPs when dispersing in fruit juice was detected by TEM and DLS measurements. Moreover, quantification of ZnONPs was achieved by using two FAAS-based methodologies. Total mass Zn in ZnONPs-spiked water was successfully determined by FAAS using ZnONPs as calibrants whereas in ZnONPs-spiked fruit juices total mass of Zn in ZnONPs was determined by FAAS using ionic Zn as calibrants. Finally, nanoparticles were successfully characterized in the three phases of an in vitro digestion assay when they were added to water and orange juice. Again a decrease in ZnONPs size was observed, especially during salivar and gastric digestion steps. The results corroborate the importance of food matrix on ZnONPs stability, bioaccessibility and toxicity. Additionally, the developed methodology evidenced the useful of FAAS technique for determining mass concentration of nanoparticles directly in samples omitting time-consuming sample treatment.

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## DISOLVENTES SUPRAMOLECULARES PARA LA DETERMINACIÓN RÁPIDA DE COCCIDIOSTATOS EN LECHE

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Los coccidiostatos ionóforos (Lasalocid, Narasin, Salinomycin, Monensin, Semduramicin y Maduramicin) son una familia de agentes antiprotozoarios obtenidos a partir de bacterias [1]. Químicamente, se caracterizan por poseer una cadena poli-éter que, de forma similar a los éteres corona, conduce a la formación de complejos con cationes monovalentes, lo que explica sus propiedades antibióticas. Actualmente, se utilizan de forma extensiva para prevenir la coccidiosis en la industria alimentaria (bovina, aviar y láctea), por lo que sus niveles residuales máximos han sido regulados por la Comisión Europea (EC124/2009, EC37/2010 y EC86/2012). Entre las diferentes matrices legisladas, la leche presenta los límites más restrictivos, con tolerancias que varían entre  $1 \text{ ng g}^{-1}$  y  $2 \text{ ng g}^{-1}$ , dependiendo del compuesto. Es por lo tanto necesario desarrollar métodos analíticos que permitan su determinación de forma rápida, económica y fiable.

Los disolventes supramoleculares (SUPRAS) están mostrando un gran potencial como alternativa verde a los métodos tradicionales de extracción [2]. Los SUPRAS son líquidos nanoestructurados bioinspirados formados por agregados de compuestos anfífilos organizados mediante autoensamblaje. Presentan regiones de diferente polaridad para la extracción de una amplia variedad de analitos. También se comportan como materiales de acceso restringido, lo cual permite realizar la extracción y exclusión simultánea de interferentes comunes en la muestra como, por ejemplo, macromoléculas (proteínas, polisacáridos, etc.).

En este trabajo se presenta el desarrollo, validación y aplicación de un método analítico basado en SUPRAS de hexanol como método rápido de extracción y limpieza para la determinación de coccidiostatos ionóforos en leche. Este disolvente, que presenta una nanoestructura hexagonal inversa, se forma de manera espontánea en la matriz acuosa de leche ( $1275 \text{ } \mu\text{L}$ ), en presencia del anfífilo hexanol ( $184 \text{ } \mu\text{L}$ ) y con la adición de un bajo volumen de tetrahidrofurano ( $84 \text{ } \mu\text{L}$ ), tras una simple etapa de extracción en vórtex (2 min, 3000 rpm) y centrifugación (15 min, 20000g) para acelerar la separación de fases. EL SUPRAS es directamente compatible con el sistema de detección por LC-MS/MS sin posteriores etapas de re-extracción, evaporación o limpieza. La recuperación obtenida fue cuantitativa para todos los coccidiostatos estudiados, obteniéndose un factor de preconcentración de 11. Los límites de detección variaron entre  $3 \text{ pg g}^{-1}$  para Salinomycin y  $28 \text{ pg g}^{-1}$  para Maduramicin, valores muy por debajo de los límites legislados.

La metodología desarrollada se aplicó al análisis de muestras de leche de diversa naturaleza y procedencia, encontrándose coccidiostatos en dos de las muestras a concentraciones que variaron entre  $23 \text{ pg g}^{-1}$  para Salinomycin y  $189 \text{ pg g}^{-1}$  para Monensin. En todo caso, cabe destacar que dichas concentraciones se encontraron por debajo de los límites establecidos por la legislación.

En conclusión, los SUPRAS son una interesante alternativa para la determinación de coccidiostatos en leche permitiendo desarrollar un método LC-MS/MS barato, rápido y de acuerdo a los parámetros establecidos por la legislación europea.

**Agradecimientos:** Los autores agradecen el apoyo financiero del MINECO (Proyecto CTQ2014-53539-R) y FEDER. D.G.G. y A.B.G. agradecen al MINECO su beca postdoctoral (FJCI-2014-20052) y Ramón y Cajal (RYC-2015-18482), respectivamente.

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# SEMI-AUTOMATED SOLID PHASE EXTRACTION SYSTEM COUPLED TO GAS CHROMATOGRAPHY-MASS SPECTROMETRY FOR THE ISOLATION AND DETECTION OF POLYCYCLIC AROMATIC HYDROCARBONS IN CEREAL BASED FOODSTUFF

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Polycyclic aromatic hydrocarbons are one of the largest families of contaminants, they have proved carcinogenic and mutagenic properties, because of this, the European Commission and the Environmental Protection Agency (EPA) included them into the priority pollutants list. Although they are included as priority pollutants they are not regulated in some families of foodstuff, in the last revision of the European Policies the PAHs has not been assessed and either limited their presence in cereal based foodstuff since the Regulation (EC) No 1881/2006, only for infants cereal based food the allowed limit is set at 1 µg/kg for benzo(a)pyrene and 1 µg/kg for the sum of benzo(a)pyrene, benzo(a)anthracene, benzo(b)fluoranthene and chrysene. The presence of this kind of compounds in cereal based foodstuff could be made by three main ways, first for the environmental pollution, caused by nature or by human activities like petrol fuel combustion, etc. that affect to the cereal crops, the use of contaminated water for the cultivation also could be a source. During the harvesting the use of lubricants in the machinery used for this purpose could be a source of PAHs for the grain, and as mentioned before, the use of fuel in those machines. Before their use in the food industry, the grains are treated under a dryness process applying heat to reduce their content in water and improve their lifetime, in some food this dryness is more intense than others, depending the destination of the grain, in the most extreme cases the grain is toasted, increasing their PAHs content [1]. In the food factory, the grains are transformed and processed into diverse kinds of food like bread, cereal, cookies, flour, pasta, rice, etc. depending of the product the use of different baking procedures could increase the PAHs levels in a significant way [2].

There is a need for the assessment and knowledge about the presence of polycyclic aromatic hydrocarbons in cereal based foodstuff and provide analytical data to help to establish new regulation policies for the allowed limits by the European Regulation. The concentration levels in those kinds of food are usually low, for that reason an analyte enrichment is required before the chromatographic determination. For solid matrices, as those products are, a homogenization process is required before the extraction procedure, the presence of lipid content must be addressed because PAHs are highly soluble in fat, and a saponification step will be required. The use of solid-phase extraction (SPE) for the sample enrichment improves the analytical sensibility while reduces the sample manipulation and the risk of being contaminated. For the PAHs determination in foodstuff samples the use of gas chromatography coupled to mass spectrometry (GC-MS) is extended and has proved their great selectivity, resolution and sensitivity [3], high resolution liquid chromatography with fluorescence detector (HPLC-FLD) has been also used over this family of compounds and had demonstrated great analytical features, but some of the PAHs has not fluorescence properties and the use of GC-MS is required [4].

The proposed methodology for the isolation and determination of PAHs in cereal grain foodstuff combining a semi-automated SPE system and GC-MS for 9 PAHs (naphthalene, anthracene, fluoranthene, chrysene, benzo[a]pyrene, benzo[b]fluoranthene, benzo[k]fluoranthene, indeno[1,2,3-cd]pyrene and benzo[ghi]perylene) obtained good analytical features, with low limits of detection, relative standard deviation (RSD less than 6%) and high recoveries (near to 100 %) in several samples of bread, cereal, cookies, flour, pasta and rice, reporting positive results for some of them.

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## DETERMINACIÓN DE TITANIO EN PRODUCTOS LÁCTEOS MEDIANTE ICP-MS

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El dióxido de titanio se emplea como aditivo alimentario (E-171) hasta concentraciones del 2%, por ejemplo, en las coberturas azucaradas de chicles o caramelos. En productos lácteos puede añadirse como espesante y blanqueante a yogures, helados, quesos, etc.

En este trabajo se ha determinado la concentración de titanio en diferentes productos lácteos. Para ello se realizó una digestión ácida asistida con microondas con ácido nítrico y sulfúrico de distintas muestras de productos lácteos y se determinó el titanio en los digeridos mediante Plasma de Acoplamiento Inductivo-Espectrometría de Masas (ICP-MS). Una de los principales problemas en el análisis del titanio en los productos lácteos es la presencia de calcio que interfiere en la determinación empleando el isótopo mayoritario  $^{48}\text{Ti}$ . Por ello se ha evaluado el empleo de otros isótopos minoritarios ( $^{47}\text{Ti}$  y  $^{49}\text{Ti}$ ), y de helio como gas de colisión para eliminar interferencias moleculares. La utilización de escandio, germanio o itrio como patrones internos no aportaron mejoras significativas en la determinación cuando se realizaron las medidas con  $^{47}\text{Ti}$  y un flujo de gas de colisión de  $1\text{ mL min}^{-1}$ .

Se estudiaron las características analíticas del método desarrollado. Los límites de detección y cuantificación obtenidos fueron de 0.05 y 0.17 mg/Kg, respectivamente. Los coeficientes de variación calculados al estudiar la reproducibilidad en las medidas fueron del 3%, y las recuperaciones analíticas medias fueron del 97%.

El método desarrollado se aplicó al análisis de una variedad de muestras de productos lácteos (leches, natas, yogures, quesos), y las mayores concentraciones de titanio (de hasta 70 mg/Kg) se hallaron en muestras procedentes de máquinas de venta automatizada de leche.

### **Agradecimientos:**

Esta investigación está financiada por el Gobierno de España (Ministerio de Economía y Competitividad, proyecto CTQ2015-68094-C2-2-R). Los autores pertenecen al Grupo de Referencia Competitivo 6RC2014/2016 (Xunta de Galicia). Estos programas están cofinanciados por los fondos FEDER (UE).

**DETERMINATION OF ULTRATRAZE LEVELS OF ALKYL METHOXY PYRAZINES IN WINE  
BY STIR BAR SORPTIVE EXTRACTION COMBINED WITH  
MULTIDIMENSIONAL GAS CHROMATOGRAPHY-MASS SPECTROMETRY**

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Alkylmethoxypyrazines (MPs) are volatile compounds present in wine at low ng/L levels. Due to their extremely low sensory detection thresholds -ng/L level-, the small concentrations naturally occurring in wine can induce off-odors (earthy, immature and peppery notes). Because of their substantial relevant sensory role, it is essential to develop highly sensitive and selective analytical methods for their determination. The need for extra selectivity is particularly important, since standard GC-MS methods for the determination of these compounds have frequently suffered high risk of false positives [1].

In this study, sensitivity is achieved by using stir bar sorptive extraction (SBSE) followed by thermal desorption (TD) as the sample preparation technique. The extra selectivity required for the analysis was obtained by applying heart-cut bi-dimensional gas chromatography coupled with mass spectrometry. Stable isotope dilution analysis was used for calibration and quantification. Extraction time, dilution factor and pH conditions were optimized to reach sufficient sensibility and reproducibility. The validated method demonstrated excellent linearity in the typical concentration range found in wine ( $R^2 > 0.9996$  for all target MPs) as well as good reproducibility (RSD below 3%). Besides, the detection limits (at sub-ppt level from 0.06 to 0.12 ng/L) were estimated to be well below the reported sensory threshold in wine. The method is robust, the instrumental determination is completely automated and it is convenient for the relatively fast analysis of batches containing numerous wine samples. It is expected that this method will make possible to further deepen the knowledge of MPs as varietal aromatic characteristics of typical wines.

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**POLYCYCLIC AROMATIC HYDROCARBONS ISOLATION AND DETERMINATION FROM  
DRINK SAMPLES BY SOLID PHASE EXTRACTION ANALYTE ENRICHMENT AND  
GAS CHROMATOGRAPHY-MASS SPECTROMETRY**

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Polycyclic aromatic hydrocarbons are a family of compounds formed by two or more fused aromatic rings, they are persistent in the environment, lipophilic and semi-volatile. The International Agency for Research of Cancer (IARC), Environmental Protection Agency (EPA) and the European Union (UE) has included them into its lists of priority pollutants. Their occurrence is caused by incomplete combustion of organic matter or thermogenic processes. Human activities like the use of fossil fuels and waste incineration cause contamination by PAHs over the environment and by consequence the crops that are used for beverage production as grapes, sugar and barley. The production process of some drinks could increase the PAHs content in them, for wine, ciders and distilled drinks the use of barrels and oak chips is required in the ageing step, the wood used has previously been exposed for a period of 1 to 3 years to the climatology and so, to the environmental pollution, and then is toasted by an open fire inside the barrels or toasting by ovens for the wood chips, this step is a major contributor to PAHs contamination. In beer and other ferments, the malting of the grain is a source of PAHs for the final product. Sugar is also toasted and dried in some drinks production, also, for some drinks the crops are burned before the harvesting, causing elevated levels of contamination in the distilled drink [1]. The main source in tea sleeves and coffee is the roasting and drying procedure for elevate the flavour and taste of those infusion drinks. In juices the use of processed sugar and the use of unpeeled fruit for the production are the main sources of contamination [2].

Despite the different and variated sources, PAHs are presented at low levels in drinks, usually in  $\mu\text{g L}^{-1}$  levels, by the data submitted by the EU countries to the European Food Safety Agency after the Commission Recommendation 2005/108/EC. This summary of data resulted useful to assess the levels of PAHs in drinks, but it hasn't been regulated yet. For liquid matrices, the extraction process could be easier compared to solid and fatty matrices, the degasification and filtration in some kinds of drinks like soft drinks, beers or juices to eliminate the solids in suspension is usually required. Thanks to the use of solid-phase extraction (SPE) for the preconcentration and analyte enrichment the analytical properties such sensibility and precision are increased while problems associated to contamination are avoided. For the determination, the use of gas chromatography coupled to mass spectrometry is widely used with good selectivity, precision and sensibility [3], high resolution liquid chromatography with fluorescence detector (HPLC-FLD) has been also used for PAHs determination with great analytical features, the disadvantage of this determination technique is that some of the analytes does not present fluorescence properties, so another determination technique is required at the same time [4].

Due to all the processes mentioned above the presence of PAHs in drinks should be assessed, the proposed methodology for the isolation and determination of PAHs in drinks combine a semi-automated SPE system and GC-MS for 9 PAHs. This method provides high precision ( $\text{RSD} < 6\%$ ), selectivity and sensitivity with low limits of detection, the methodology has good recoveries reaching near 100 % for the studied PAHs in several samples of beer, distilled drinks, soft drinks, tea, coffee, wine, cider and juices.

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# EVALUATION OF THE PRESENCE OF TROPANE ALKALOIDS IN CEREALS AND LEGUME MATRICES USING LIQUID CHROMATOGRAPHY COUPLED TO HIGH RESOLUTION MASS SPECTROMETRY

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Buckwheat and other matrices as soy, linseed and millet are being more consumed because their antidiabetic, hypotension, hypocholesterolemic and hypoglycemic effects [1]. However these matrixes can be contaminated with others plants as *Solanaceae* family plants that have anticholinergic effects [2]. There are more than 200 toxic compounds belonging to tropane alkaloids family, although the most studied are atropine and scopolamine. That is why, EFSA [2] and the European Commission [3] recommend the study of a larger range of tropane alkaloids in food matrixes like cereal and legume.

The purpose of this study is the development and validation of a method that allow the quantification of a larger range of tropane alkaloids in buckwheat, linseed, millet and soy. The tropane alkaloids studied were anisodamine, scopolamine, atropine, littorine, aposcopolamine, homatropine, apoatropine, cuscohygrine, pseudotropine, physoperuvine, tropine, scopoline, tropinone and tropane. A simple solid-liquid extraction was employed, using a methanol:water 0.5% acetic acid (2:1, v/v) mixture as extraction solvent. Then a preconcentration and clean-up stage was used by a strong cation exchange solid phase extraction. The compounds retained in the cartridges were eluted utilizing methanol containing 3% of ammonium hydroxide solution (25%). The extract was evaporated and reconstituted in methanol:water 50:50 (v/v). The analysis was performed by high pressure liquid chromatography coupled to mass spectrometer Orbitrap analyzer (HPLC-MS-Orbitrap). The chromatographic method used two coupled columns to get a suitable chromatographic separation (Zorbax Eclipse Plus C18 and Zorbax Eclipse Plus HILIC).

The method was validated obtaining recoveries in the range of 60-109% (except for some compounds in soy), precision values (expressed as relative standard deviation) lower than 20% and quantification limits lower than 3 µg kg<sup>-1</sup>. Finally, the method was applied to real samples, obtaining positives samples for buckwheat (below the quantification limits), and millet, detected scopolamine at 23 µg kg<sup>-1</sup>. Furthermore, samples contaminated with *Datura Stramonium* and *Brugmansia Arborea* were analyzed obtaining concentrations up to 1800 µg kg<sup>-1</sup> for atropine in samples contaminated with *Stramonium* seeds.

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*The authors acknowledge Ministry of Economy and Competitiveness (MINECO) and European Regional Development Fund (ERDF) (project ref. CTQ2015-69899-R) the financial support.*

# DETERMINATION OF HORMONES IN SEVERAL TYPES OF MEAT BY LIQUID CHROMATOGRAPHY-ORBITRAP HIGH RESOLUTION MASS SPECTROMETRY

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Hormones are needed to control and regulate the activity of specific cells or organs. They are chemical substances produced in the body, although synthetic hormones could have deleterious effects in human health [1]. They can be divided into steroid and non-steroid hormones. Steroid hormones are a group of lipid molecules that can be classified into glucocorticoids, mineralocorticoids, androgens, estrogens and progestogens [2]. Estrogens are used as contraceptives, in hormonal therapies and treatment for certain types of cancer, whereas glucocorticoids have been widely prescribed for a wide range of therapeutic applications such as severe allergies, skin problems and arthritis. They are also used as growth promoters in certain farming practices [3]. Many evidences have indicated that the steroid hormones and their metabolites in animal origin food are potentially toxic and have carcinogenic effect on human health. Moreover the uses of these compounds have been restricted and banned in Europe [4]. For the determination of those compounds, the use of high resolution Exactive-Orbitrap mass analyzer offers multiple advantages owing to its sensitivity and its ability to analyze an unlimited number of compounds, performing accurate mass measurements combined with high resolving power [5].

In the present work an analytical method has been developed and validated for determining 20 hormones, belonging to the glucocorticoids, androgens, estrogens and progestogens chemical classes, and their metabolites in three types of meat samples (chicken, pork and beef) by liquid chromatography (LC)-Orbitrap. Each matrix was extracted comparing two different methods, QuEChERS (Quick, Easy, Cheap, Effective, Rugged and Safe) and "dilute and shoot". The best results were obtained with the QuEChERS method with subsequent cleaning of the extract by various combinations of sorbents (PSA, magnesium sulfate, florisil, basic alumina and C18). The most useful cleanup was obtained when 100 mg of a mixture of florisil:basic alumina (50:50, w/w) was used. Validation was performed and according to SANTE guidelines [6] satisfactory validation parameters were obtained. Such parameters were linearity, recovery (ranged between 70 to 120%), precision (repeatability and reproducibility) (obtained values < 20%), limit of identification (LOI) (for confirmation purposes) and limit of quantification (LOQ) (in the range of 1-5 µg kg<sup>-1</sup>). Matrix-matched standard solutions were used to eliminate the matrix effect. The slopes of each matrix were compared and similar slopes were obtained for chicken and pork, and one of them can be used as representative matrix of these two matrices. The applicability of the method was proved analyzing several samples belonging to the three types of meat.

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**CORRELATION OF ANTIOXIDANT PROPERTIES OF SEED PROTEIN HYDROLYSATES OBTAINED FROM DIFFERENT *OLEA EUROPAEA* AND *PRUNUS PERSICA* GENOTYPES WITH THEIR PEPTIDE COMPOSITION DETERMINED BY RP-HPLC-ESI-Q-TOF**

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Presence of reactive species (RS) is unavoidable and they play deleterious roles in proteins, DNA (deoxyribonucleic acid) and RNA (ribonucleic acid) molecules, sugars and lipids. Control of RS presence in living organisms depends on their generation and on their elimination being necessary a suitable balance. Nevertheless, under certain conditions (smoking, environmental pollutants, radiation, drugs, pesticides, industrial solvents, ozone, diseases, stress, etc.), an imbalance in these processes can provoke oxidative stress. In order to compensate this situation, the consumption of antioxidant compounds is recommended.

Negative consumer perception to synthetic antioxidants has urged the exploration of natural compounds with antioxidant properties. Especially interesting are antioxidant peptides obtained from cheap and sustainable sources such as food industry byproducts. Indeed, antioxidant peptides have been extracted from different waste materials [1, 2]. Among them, fruit seeds are promising sources of antioxidant peptides but no previous work has studied the effect of the genotype on these properties. This work studies the antioxidant properties of different olive and peach genotypes and relates them with the peptides identified by tandem mass spectrometry.

Proteins were extracted from different olive and peach seeds (five and ten varieties, respectively) and they were hydrolyzed with Alcalase enzyme to obtain peptides. All genotypes showed peptide contents ranging from 2.1 to 3.2 mg/mL. Antioxidant capacity was explored using four different *in vitro* assays (hydroxyl radical inhibition, ABTS radical scavenging, reduction of Fe (III), lipid peroxidation inhibition) and was confirmed by flow cytometry analysis. All varieties presented antioxidant properties although they significantly differed among them. *Cornicabra* olive seeds and the commercial nectarine highlighted like genotypes with the best antioxidant properties. Moreover, cytotoxicity studies demonstrated high cell viability in normal HK2 cells. Finally, all peptides were identified by RP-HPLC-ESI-Q-TOF and sequenced by *de novo* using PEAKS software. Eighteen common peptides were detected among all olive seeds while only two common peptides were observed among peach seeds. Especially interesting were specific peptides identified for most active varieties.

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**EVALUATION OF DIFFERENT DIRECT MASS SPECTROMETRIC METHODS FOR THE CHARACTERIZATION OF VIRGIN OLIVE OIL**

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Olive oil represents a key ingredient in the Mediterranean diet, having high impact on health and wellness of population, due to their contribution to prevent many relevant diseases. A vegetable oil presents a complex matrix mainly composed by triacylglycerides and other minor compounds such as free fatty acids, phosphatides, hydrocarbons or fatty alcohols, being their distributions a characteristic of different types of vegetable oils. Amongst olive oils, those with high market prize, such as virgin olive oils (VOO) may be adulterated with lower priced oils (i. e. seed or refined oils). Thus, ensure the quality and authenticity of olive oils, is an important task from both economic and health point of view. This involves a continuous need to improve analytical methods to determine the origin, quality and possible adulterations of olive oils.

Ideal analytical methods require minimum sample preparation, rapid and accurate analysis, and straightforward automatization. Vegetable oils can be characterized by optical techniques such as fluorescence and vibrational spectroscopy, mass spectrometry and also other spectrometric techniques such as nuclear magnetic resonance spectrometry. Likewise, ambient mass spectrometry ionization (AMSI) is an alternative, which is continuously growing, since minimal to none sample treatment is required, allowing the acquisition mass spectra using ions created outside the instrument without any requirement of vacuum. The aim of this work is to study different approaches for direct olive oil analysis for quality control purposes. The tested methodologies include, the use of electrospray ionization (ESI) (direct infusion) or AMSI sources such as low-temperature plasma (LTP) or paper spray (PS). In order to avoid mass spectrometer contamination, direct infusion studies were performed using olive oil diluted with appropriate solvents at different ratios. On the other hand, samples for AMSI sources were used without sample treatment (untreated samples) or minimum sample treatment such as a simple dilution. Other sample treatments such as quick liquid-liquid extraction were also tested in order to obtain a fraction enriched in a specific part of the olive oil composition (i.e. polyphenol rich fraction or lipid/fatty acid profile). Main components identified were different classes of phenolic acids and polyphenols, which are present in virgin olive oils in the mg per liter level.

**Acknowledgements**

The authors acknowledge funding support from Regional Government of Andalusia (Spain) "Junta de Andalucía" (Research Group FQM-323 and Projects Refs. AGR-6182 and FQM-2242).

**DETERMINATION OF PESTICIDE PROCESSING FACTORS DURING VIRGIN OLIVE OIL PRODUCTION**

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The establishment of Maximum Residue Levels (MRLs) of pesticides in raw and processed food is a policy necessary to ensure food safety and thus, to protect consumer health. However, although the procedure to set MRLs in raw food is standardized, the definition of MRLs in processed food is often a matter of discussion. In Europe, a decade after EU regulation 396/2005 became into force, annex VI on MRLs in processed food remains unpublished. Considering this scenario, the purpose of the present work was the experimental evaluation of processing factors of a large set of representative pesticides applied in olive orchards worldwide. The ultimate goal is to understand their behavior during olive oil production and predict their processing factors, which could be eventually used for the calculation of MRLs in olive oil from the MRLs set in olives. A laboratory-scale Abencor system was used for the production of olive oil from olives spiked with the 104 pesticides studied, being three different chromatographic methods used for the analysis of raw olives and the obtained olive oil: (i) gas chromatography tandem mass spectrometry (GC-MS/MS) for GC-amenable pesticides; (ii) hydrophilic interaction liquid chromatography tandem mass spectrometry (HILIC-MS/MS) for polar pesticides, and; (iii) reversed phase liquid chromatography tandem mass spectrometry (UHPLC-MS/MS) for low to medium polarity pesticides. The behavior of the pesticides during olive oil extraction was plotted against their polarity using Kow values, finding a linear relation between the processing factors experimentally calculated and their octanol water partitioning coefficient (log Kow). With this relation set, the processing factors of new pesticides could be estimated and, additionally, an accurate estimation of the actual concentration of pesticides transferred from olives to olive oil could be also made, enabling the calculation of the equivalent MRLs in olive oil from the MRLs in olives, considering the percentage of oil extracted (oil yield) and the log Kow of each pesticide.

**Acknowledgements**

The authors acknowledge funding support from Regional Government of Andalusia (Spain) "Junta de Andalucía" (Research Group FQM-323 and Project Ref. AGR-6182). Technical support provided by Dr. Juan Castro-Marmol from CICT (Centro de Instrumentación Científico-Técnica) of the University of Jaén is gratefully acknowledged. D.M.G. thanks the Spanish Ministerio de Economía y Competitividad (MINECO) for a *Juan de la Cierva Formación* postdoctoral contract (ref. FJCI-2014-19573).

**EVALUATION OF A MODIFIED QuEChERS METHOD FOR THE MONITORING OF MYCOTOXIN RESIDUES IN NUTS BY NANO FLOW LC-HRMS**

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Mycotoxins are toxic secondary metabolites produced by a wide range of fungi growing on many agricultural commodities and processed food. Their presence in food could show mutagenic, teratogenic, carcinogenic, and immunosuppressive effects in animals and humans. In fact, several organizations including European Union have regulated the levels of mycotoxins in crops such as nut, cereal, corn, milk, and edible oils. For instance, the maximum residue level for aflatoxin B1 in peanut is 8  $\mu\text{g kg}^{-1}$ . Thus, the development of analytical methods, which can determine mycotoxin residues in these types of matrices at very low concentrations, has acquired significant relevance across the globe. Nano flow liquid chromatography coupled with electrospray tandem MS can be an interesting alternative to conventional LC methodologies, since it provides significant benefits in terms of sensitivity and matrix effect reduction. In this work, a new analytical method based on nanoflow liquid chromatography high resolution mass spectrometry is proposed for the identification and simultaneous quantification of a representative group of mycotoxins in nut samples. Detection was undertaken with a Thermo Q-Exactive Orbitrap mass spectrometer equipped with an Easy-Spray nano-electrospray ion source. For the separation of the studied analytes, a Thermo Scientific EASY-nLC 1000 nano-LC system was used. An EASY-Spray column (PepMap®, C18, 3  $\mu\text{m}$ , 100Å, 75  $\mu\text{m}$  x 150 mm) was employed. Mobile phases A and B were water and acetonitrile, respectively, both with 0.1 % formic acid. The injection volume was 1  $\mu\text{L}$ . Flow rate was set at 300  $\text{nL}\cdot\text{min}^{-1}$ . Another aspect of paramount interest for the determination of mycotoxin in nut samples is the sample treatment. It is not an easy task due to the high lipid composition of this type of samples, requiring sample treatments with exhaustive clean-up. QuEChERS methodology has been proposed as a straightforward alternative for extraction and clean-up. Moreover, a new sorbent, Enhanced Matrix Removal-Lipid (EMR-Lipid) has been tested for dispersive solid phase extraction (dSPE), after extraction of mycotoxin with acetonitrile. Preliminary studies have yielded promising results in terms of matrix effect, sensitivity and recovery, obtaining negligible matrix effects for all mycotoxins studied in peanut, pistachio and almond. Thus, nano flow LC-HRMS combined with a modified QuEChERS based on the use EMR-Lipid could be used as a, convenient and high throughput method for clean-up and analysis of mycotoxin in nut samples at trace concentrations.

**Acknowledgements**

The authors acknowledge, funding support from Ministerio de Economía y Competitividad (MINECO) (Ref. CTQ-2015-71321-P). D.M.G. thanks the Spanish Ministerio de Economía y Competitividad (MINECO) for a *Juan de la Cierva* Formación postdoctoral contract (ref. FJCI-2014-19573).

**DETERMINATION OF TROPANE ALKALOIDS IN CEREAL-BASED FOODS  
USING QUECHERS WITH MIXED-MODE SPE CLEANUP FOLLOWED BY  
LIQUID CHROMATOGRAPHY TANDEM MASS SPECTROMETRY**

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The recent introduction of the Commission Regulation (EU) 2016/239 on tropane alkaloids in certain cereal-based foods has opened two challenges. The maximum level of 1 ng g<sup>-1</sup> for scopolamine and atropine has been set to the strict level of prohibited pesticides, which calls for highly selective and sensitive analytical methods. On the other hand, for the food and feed industry, the valorization of contaminated batches and raw materials is an unsolved issue, except for the evident cases of deposition and pellet preparation for heating. Soil amelioration might be the preferred choice, however, the data on soil persistence and uptake by plants are scarce and somehow controversial.

The goal of our study was: (i) to develop analytical methods for the quantification of atropine and scopolamine in the regulation-related food matrices and (ii) to monitor the degradation of tropane alkaloids in naturally contaminated millet waste in a soil experiment. To meet the current MRLs, a modified QuEChERS method with/without mixed mode SPE assistance was validated for millet and millet based products. Selective and sensitive analytical approach was targeted with two different UHPLC-ESI-QQQ-MS with analytical and narrowbore C18 columns, respectively. Facing the lack of CRMs certified for tropane alkaloids, recovery studies were conducted on the soil and millet samples. Concerning selectivity, the careful choice of MRM parameters was especially important for the soil experiment due to the interference on the most used transitions. The requested analytical performance of LOQ < MRL for the alkaloids could be directly achieved only by one of the instruments using an analytical C18 column (LOQs: 0.1-0.4 ng g<sup>-1</sup>). In the case of the second instrument (narrow bore C18 column), the use of mixed mode SPE after the QuEChERS step was required to decrease the LOQ of 5.0 ng g<sup>-1</sup> to 0.4 ng g<sup>-1</sup>. The soil experiments could be concluded with the observation that both tropane alkaloids were degraded within two weeks, which definitely opens a viable valorization path.

**Acknowledgements**

The authors acknowledge funding support from Regional Government of Andalusia (Spain) "Junta de Andalucía" (Research Group FQM-323). D.M.G. thanks the Spanish Ministerio de Economía y Competitividad (MINECO) for a *Juan de la Cierva Formación* postdoctoral contract (ref. FJCI-2014-19573).



**DETERMINACIÓN DE SULFAMIDAS EN MIELES DE LA COMUNIDAD AUTÓNOMA DE EXTREMADURA****M. Palomino-Vasco, I. Márquez Lianes, M.I. Rodríguez Cáceres, M.I. Acedo-Valenzuela**

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Las sulfamidas son conocidas desde principios del siglo XX. Actúan como agentes bacteriostáticos y poseen actividad contra infecciones causadas por bacterias Gram-positivas y Gram-negativas y algunos protozoos. Solían emplearse en apicultura. El método más utilizado para la prevención de enfermedades, era alimentar a las abejas con una cierta cantidad de sulfamidas en invierno o a principios de primavera para aumentar su inmunidad. En algunos países se han establecido límites máximos de residuos. Así por ejemplo, en Bélgica y Reino Unido están fijados en 20 y 50 ng/g para las sulfamidas totales en miel y en Suiza 50 ng/g, refiriéndose a la suma de sulfamidas y sus metabolitos [1]. Actualmente, en la normativa europea (Reglamento (CEE) No 2377/90 del Consejo) no aparece los límites máximos de residuos en el uso de sulfamidas en miel, por los que podría deducirse que su uso en la apicultura está prohibido.

La apicultura en Extremadura adquiere su mayor importancia en enclaves abruptos o con suelos de poca calidad y, por tanto, no explotados agrícolamente, donde predomina la vegetación mediterránea [2]. Solo contamos con la Denominación de Origen Protegida "Miel de Villuercas-Ibores".

El objetivo de este trabajo es comprobar que las mieles extremeñas no contienen residuos de sulfamidas. El método analítico habitual de determinación de estos analitos es la cromatografía con detección fluorescente. Como no presentan fluorescencia nativa, es necesario realizar una reacción de derivatización. El derivatizante empleado fue fluorescamina, que es un reactivo no fluorescente que sirve para la detección de aminas primarias, péptidos y proteínas.

Para llevar a cabo la determinación, se empleó un método cromatográfico encontrado en la bibliografía [3], pero fue necesario modificar el gradiente de elución para que las 7 sulfamidas estudiadas (Sulfatiazol; Sulfametazina; Sulfametoxipiridazina; Sulfisoxazol; Sulfadoxina; Sulfametoxazol; Sulfadimetoxina) estuvieran separadas. En las condiciones optimizadas, todos los picos salían antes de 40 minutos. Se estudió la relación entre señal analítica y concentración por los métodos de patrón externo y patrón interno, empleando sulfameter como patrón interno. Se determinaron así mismo los límites de detección por el método de Clayton, estando éstos comprendidos entre 2,8 ppb (sulfametazina) y 22 ppb (sulfadoxina). Se realizó un estudio de la repetitividad intraday e interday, obteniendo RDS aceptables, siempre menores al 15%, siendo más bajos en el intraday que en el interday.

De las 15 mieles analizadas, en ninguna de ellas se encontraron restos de las sulfamidas estudiadas en este trabajo. Sí se comprobó que en algunas de ellas aparecía un pico desconocido en torno al minuto 27, que no era ninguno de los analitos objetos de estudio. Así mismo se comprobó que no se correspondía con otras sulfamidas disponibles ni con aminas biógenas, que también podrían estar presentes en la miel.

**Agradecimientos.** Los autores agradecen la financiación de este trabajo al Ministerio de Economía y Competitividad de España (Proyecto CTQ2014-52309-P) y a la Junta de Extremadura (IB16058 y GR15090-Grupo de investigación FQM003). M. Palomino-Vasco agradece a la Junta de Extremadura la concesión de su contrato predoctoral (PD16033). Todos los proyectos están cofinanciados por los Fondos Europeos FEDER.

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**MULTI-MYCOTOXIN ASSESSMENT IN COMMERCIAL FEEDS BY COMBINING  
UPLC–ESI–MS/MS AND UPLC–QTOF–MS TECHNIQUES**

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An optimized and validated UPLC–ESI–MS/MS method for the simultaneous determination of aflatoxins, ochratoxin A, zearalenone, deoxynivalenol, fumonisins B1 and B2, T-2 and HT-2 toxins, fusarenone X, diacetoxyscirpenol, and 3- and 15-acetyldeoxynivalenol in feedstuff samples has been developed. A quadrupole-time-of-flight mass spectrometer detector (QTOF–MS) operating in full scan mode was combined with the UPLC system to identify other possible microbial metabolites occurring in compound feed samples and to confirm mycotoxins previously detected by UPLC–ESI–MS/MS. Sixty-two commercial feed samples from the Spanish market were analyzed. Metabolite extraction was performed with acetonitrile-water-formic acid (80:19:1, v/v/v). No further cleanup by immunoaffinity column was needed and matrix-matched calibration was performed. Method detection and quantification limits and performance criteria set by Commission Regulation (EC) No 401/2006 were fulfilled. Relatively high levels of the main regulated mycotoxins were found in feed samples although they did not surpass the limits set by the European regulations. Using UPLC–QTOF–MS, many non-regulated mycotoxins and other fungal/microbial metabolites were identified on the basis of accurate mass measurements of the main quasimolecular ions. Some of them were also identified by their high purity score. This is a pioneer study to assess contamination of feed with multiple mycotoxins and other microbial metabolites using a combination of UPLC–MS/MS and UPLC–QTOF–MS.

**Acknowledgements:** The authors wish to thank financial support from FEDER and “Ministerio de Economía y Competitividad” (MINECO, Spanish Government) (Project AGL2014-53928-C2-1-R). E.M. Mateo is grateful to MINECO for a “Juan de la Cierva” postdoctoral contract (Ref. FJCI-2015-25992). J.V. Gómez is grateful to MINECO for a PhD contract (BES-2015-071242).

## STUDY OF THE CONTAMINATION OF SPANISH BARLEY SEEDS WITH AFLATOXINS AND OCHRATOXIN A

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Barley is a nutritious cereal that contains carbohydrates, proteins, vitamins and minerals among other nutrients. It is the fourth largest cultivated cereal crop in the world and the most important cereal crop in Spain. Barley kernels can be contaminated with toxigenic fungal species and mycotoxins, which causes quality and nutritional losses and embodies a significant hazard to the food chain. To date, hundreds of mycotoxins have been isolated and described, among them aflatoxins B1 (AFB1), B2 (AFB2), G1 (AFG1) and G2 (AFG2) and ochratoxin A (OTA) have been found to occur in barley and other cereals and by-products in different countries. Aflatoxins are a group of structurally related toxic secondary metabolites produced mainly by certain strains of *Aspergillus flavus* and *A. parasiticus*. In barley, they are perhaps the most important toxigenic species. AFB1 is predominant and the most toxic and most potent hepatocarcinogenic natural compound ever characterized. Ochratoxins are a group of structurally related secondary fungal metabolites being OTA the most toxic member of the group. The most important ochratoxigenic *Aspergillus* species are *A. carbonarius*, *A. ochraceus* and other xerophilic similar species, *A. westerdijkiae* and *A. steynii*. OTA exhibits teratogenic, embryotoxic, genotoxic, neurotoxic, carcinogenic (group 2B), nephrotoxic and immunosuppressive effects.

Due to the paramount importance of barley and its by-products in the diet of humans and animals, the aim of this study was to assess the occurrence of AFB1, AFB2, AFG1, AFG2 and OTA in stored barley grain in Spain using a previously optimized and validated method. It involved accelerated solvent extraction using an acetonitrile-water (60/40 v/v) mixture, immunoaffinity column cleanup, reversed-phase HPLC separation using a gradient-programmed mobile phase, post-column derivatization with iodine to enhance fluorescence intensity of AFB1 and AFG1, and scanning fluorescence detection. One hundred and five barley seeds samples collected from Spanish grain stores and silos were analyzed. Twenty-nine samples were contaminated with at least one of the mycotoxins under study. AFB1, AFB2, AFG1, AFG2, and OTA were detected in 12.4%, 2.9%, 4.8%, 2.9%, and 20% of the samples, respectively. Aflatoxins and OTA co-occurred in 4.8% of the samples. The highest mycotoxin levels (ng/g) found were 0.61 (AFB1), 0.06 (AFB2), 0.26 (AFG1), 0.05 (AFG2), and 1.6 (OTA). They were below the maximum limits established by the European regulations for this kind of commodity.

**Acknowledgements:** The authors wish to thank financial support from FEDER and "Ministerio de Economía y Competitividad" (MINECO, Spanish Government) (Project AGL2014-53928-C2-1-R). E.M. Mateo is grateful to MINECO for a "Juan de la Cierva" postdoctoral contract. (Ref. FJCI-2015-25992). J.V. Gómez is grateful to MINECO for a PhD contract (BES-2015-071242).

## DIRECT METHODOLOGIES FOR THE DETERMINATION OF MINERAL CONTENT IN FOOD SAMPLES.

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In the present work, the viability of the use of X-Ray fluorescence equipment (XRF) for the determination of the mineral content in cocoa powder and hot chocolates has been studied. The XRF allows the rapid multi-elemental quantification of elements. For the validation of the results obtained by the XRF, they were compared with those obtained by ICP-OES, an instrumental technique perfectly contrasted as reference method technique.

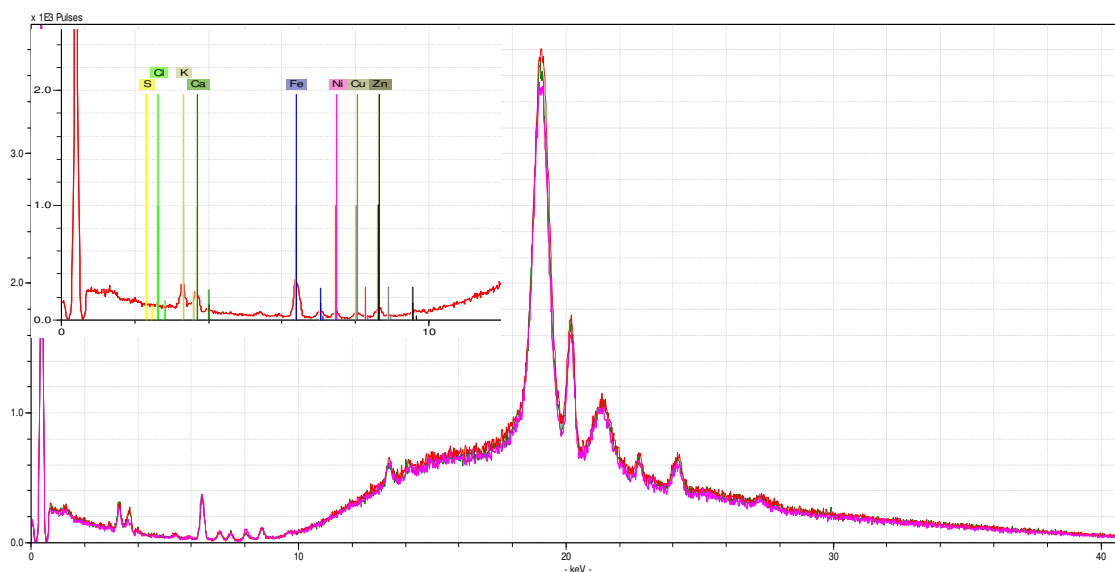
From comparison of results, it could be concluded that the internal calibration of the XRF (GeoChem and Low Density) cannot accurately quantify the analytes in the food matrices, so for that, series of calibrations were performed using samples of cocoa as "standards", diluted with base sugar and using the intensity of the XRF in front of the concentrations of the analytes determined employing ICP-OES.

For the direct measurement of the samples by XRF, a series of pills of 13 millimeters in diameter and 2-3 millimeters of thickness corresponding to approximately 0.8 g of pure samples of cocoa powder and dilutions of these with the sugar (sucrose or glucose), were employed. The samples were digested in a microwave oven and carried to a suitable volume to obtain the signal in the linear range to quantify the mineral content by ICP-OES.

In order to develop a calibration curves useful for any cocoa samples several types of cocoa powder were employed.

The signal intensities obtained by XRF interpolated in the cocoa calibration provides mineral contents agree with those found by ICP-OES.

Additionally, the developed external calibrations have been applied to the quantification of the mineral content in infantile powdered milk with very satisfactory results.



Authors acknowledge the financial support of the Generalitat Valenciana, Project PROMETEO II 2014/077.

**RAPID METHOD FOR THE DETERMINATION OF ANTHELMINTIC BENZIMIDAZOLES AND METABOLITES IN MILK BY UPLC-MS/MS****M. Bustamante-Rangel, M. M. Delgado-Zamarreño, E. Rodríguez Gonzalo**

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Benzimidazoles are anthelmintic agents widely used in the prevention and treatment of parasitic infections in animals destined for human consumption. In general, it can be proposed that anthelmintic residues pose no human health risk if veterinary drugs are properly administered and the recommended doses are correctly met. However, the extensive use of these substances can leave residues in edible animal products, which may have negative effects on the human health such as congenic malformations, teratogenicity, diarrhoea, anaemia, pulmonary edemas, etc. For these reasons, the European Union has set maximum residue limits (MRLs) for benzimidazoles and their metabolites in foodstuffs of animal origin (European Commission Regulation 37/2010 [1]). The MRLs listed for anthelmintic benzimidazoles in milk include the albendazole, fenbendazole and thiabendazole families.

In this work, a simple and rapid method based on LC-MS/MS has been developed for the analysis of these families of benzimidazoles in milk. The use of a Phenomenex 100 x 2.1 mm Kinetex® 2.6  $\mu$ m EVO C18 column allowed for a significant reduction in the analysis time with respect to conventional columns [2]; separation of the analytes was achieved in 10 min. Extraction of the analytes from the samples was achieved using the QuEChERS method, based on a salting out assisted solvent extraction followed by dispersive solid-phase extraction (d-SPE). Acetonitrile and PSA have been used as the extraction solvent and the sorbent for d-SPE, respectively.

The parameters evaluated for the validation of the developed method included linearity, matrix effect, detection and quantification limits, repeatability, reproducibility and accuracy. The validated method exhibited linearity with correlation coefficient ( $R^2$ ) higher than 0.9990 in the range of 0.5 to 2.0 times the maximum residue limit (MRL). Detection limits ranged from 0.77 to 3.3 ng kg<sup>-1</sup> for oxfendazole (OFZ), albendazole (ABZ), fenbendazole (FBZ) and febantel (FBT) and from 11 to 115 ng kg<sup>-1</sup> for the rest of benzimidazoles; quantification limits were in the 2.6-11 ng kg<sup>-1</sup> range for OFZ, ABZ, FBZ and FBT and in the 38-383 ng kg<sup>-1</sup> range for the rest of benzimidazoles. Repeatability (4.6-13 %), reproducibility (4.6-17%) and accuracy (88-119 %) offered satisfactory results for the analytes studied.

The optimized method was successfully applied to five different commercial milk samples (whole milk, skimmed milk, semi-skimmed milk and goat's milk), and the recoveries obtained at three concentration levels varied between 73 and 132 % for the lowest concentration and between 94 and 120 % for the highest level.

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**DETERMINACIÓN CINÉTICO-ESPECTROFOTOMÉTRICA DE COMPUESTOS NITROGENADOS EN VINOS DE LA D.O. RIBERA DEL GUADIANA****N.M. Mora Díez, M.I. Rodríguez Cáceres, M.J. Murillo Romero**

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La seguridad en los alimentos es una de las mayores preocupaciones de los países desarrollados, eso, unido en el caso del vino, a un mercado cada vez más competitivo y exigente en tema del control sanitario, hacen que la presencia de compuestos nitrogenados, concretamente aminos biogénos pueda llegar a ser un problema cada vez mayor. Estas aminos se encuentran presentes en alimentos y bebidas fermentadas. En el caso del vino, la determinación de aminos biogénos, es de especial interés, además de por sus efectos tóxicos, por razones de impacto sensorial negativas. Suiza ha establecido un máximo de histamina en vinos de 10 mg/L. Otros países han recomendado cantidades inferiores o iguales a 10 mg/L [1]. Por lo general, el contenido de aminos es superior en la mayoría de los alimentos que en los vinos, sin embargo, es muy importante controlar el contenido en vino, porque el etanol inhibe la metabolización intestinal de éstas, potenciando su acción tóxica.

Por su estructura, estos analitos son difíciles de determinar por métodos directos, por ello es necesario recurrir a la derivatización. Por ello, en primer lugar, se estudió, mediante fluorescencia molecular la reactividad entre putrescina (como modelo de amina biogénica) y el reactivo derivatizante 1,2-naftoquinona-4-sulfonato sódico (NQS). Se comprobó que el reactivo era inestable en las condiciones de trabajo, por lo que se decidió trabajar con métodos cinéticos. Se trabajó en cubeta y en medio básico, adicionando los reactivos en el orden, disolución reguladora – agua – HCl – putrescina y en último lugar NQS, para evitar la descomposición de éste en medio básico. Una vez registradas las curvas cinéticas, se emplearon dos métodos de medida, el de velocidad inicial (en el tramo lineal comprendido entre 33 y 59 segundos) y método integral a tiempo fijo, en ambos casos se tomaron dos medidas a 342 y 465 nm. En las condiciones optimizadas se ha propuesto un nuevo método cinético-espectrofotométrico para la determinación de putrescina, utilizando como señal analítica la pendiente de las curvas cinéticas y la diferencia de absorbancia ( $A_{59}-A_{33}$ ). El método es lineal entre 1,3-8,8 mg/L y tiene un límite de detección de Clayton comprendido entre 3,24 y 4,30  $\mu\text{M}$  en función de la longitud de onda y de la señal analítica empleada.

A continuación se procedió a aplicar el método desarrollado a muestras de vino sintético, obteniéndose recuperaciones satisfactorias comprendidas entre el 80 y 104%. Finalmente, se analizaron vinos de la D.O. Ribera del Guadiana sin necesidad de tratamiento previo de las muestras, solamente por dilución (1/10 para vinos blancos y 1/50 para tintos). Los valores de concentraciones encontrados son superiores en tintos que en blancos y los menores valores se obtienen cuando se registran las curvas cinéticas a 465 nm y se utiliza la diferencia de absorbancia como señal analítica.

A la vista de los resultados obtenidos, se puede observar que la concentración de compuestos nitrogenados encontrados en vinos blancos oscila entre 64 y 108 mg/L cuando se utiliza la pendiente como señal analítica y entre 22 y 130 mg/L cuando lo que se utiliza es la diferencia de absorbancia. Estos datos están en concordancia con el rango que se puede encontrar en la bibliografía [2,3] para la suma de aminoácidos y aminos biogénos.

**Agradecimientos.** Los autores agradecen la financiación de este trabajo al Ministerio de Economía y Competitividad de España (Proyecto CTQ2014-52309-P) y a la Junta de Extremadura (Proyectos IB16058 y GR15090-Grupo de investigación FQM003). Todos los proyectos están cofinanciados por los Fondos Europeos FEDER.

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## BIOAVAILABILITY OF TRACE AND ULTRATRACE METALS IN CHILEAN 'NATURAL WINES' FROM ITATA VALLEY

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Itata Valley, located in the South Valley, comprises an area bordered by the Andes and a lower coastal mountain, and it is one of the most important winemaking areas in Chile. Current trends regarding winery practices imply the production of 'natural wines', wines made without chemical and minimum technological intervention in growing grapes, and also in winery practices. Chemical characterization of these wines is scarce, and data regarding bioavailability of essential, and also toxic compounds, are not available.

The aim of the current work has been the assessment of the bioavailable fraction of trace and ultratrace metals in Chilean 'natural wines'. An *in vitro* bioavailability approach based dialyzability has been applied. The *in vitro* digestion procedure consisted of two sequential stages, which simulate gastric and intestinal digestion. The first step requires 0.15 g of a freshly prepared gastric solution [6.0 % (m/v) pepsin in 6.0 M hydrochloric acid], and incubation at 37 °C with an orbital – horizontal shaking at 150 rpm for 120 min. The second step uses 5.0 mL of intestinal solution (4.0 % (m/v) pancreatin and 2.5 % (m/v) bile salts dissolved in 0.1 M sodium hydrogen carbonate), and also incubation at 37 °C with an orbital – horizontal shaking at 150 rpm for 120 min]. During this second stage, dialysis membranes (10 kDa cut-off) filled with 20 mL of 0.15 N PIPES (pH 7.5) were used for simulating bio-absorption. Total concentrations of trace and ultratrace metals in wines were directly assessed by inductively coupled plasma – mass spectrometry (ICP-MS) after 1:10 dilution with 1.0 % (v/v) nitric acid. Similarly, dialysates analysis has also been performed by ICP-MS after 1:5 dilution in ultrapure water. Bio-availability ratios (dialyzability) were finally obtained by calculating the percentages of total metals concentrations in the PIPES solution inside the dialysis membranes (bio-available fraction), and those found in wines.

In general, moderate dialyzability ratios were assessed in most of wines (fifteen red wines, and three white wines). Li bioavailability was within the 38-77% range; whereas, Ba and B bioavailability ratios were from 21% to 58% (Ba), and from 31% to 50% (B). Mn dialyzability varied from 17% to 27%, and ratios from 9% to 30% were found for Sr. Major metals, such as Mg, Ca and K, showed ratios within the 16-27%, 11-36%, and 27-43% ranges, respectively. Low bioavailability was observed for Al (from 3% to 11%), and for Fe (from 1% to 17%). Regarding Cu, Co, and Sn, most of dialyzates contained un-detectable concentrations. Only one wine sample offered a dialyzability ratio of 36% for Cu, and Co was quantified in two dialyzates (16 and 18% of dialyzability). Sn was present in eight dialyzates (bioavailability ratios from 5 to 65%). Other metals such as Ag, As, Be, Cd, Cr, Hg, Mo, Ni, Pb, Pt, Se, Sb, Ti, Tl, V, and Zn, were not bioavailable (the concentration in dialyzates was lower than the limit of detection of the methods). Most of these metals (Be, Cd, Cr, Mo, Ni, Pb, Sb, Ti, Tl, V, and Zn) were, however, present in all analysed wine samples. Other metals such as Ag, As, Hg, and Pt were quantified in few wines samples: Ag (three wines samples), As (seven wine samples), Hg (one wine sample), and Pt (four wine samples). Se was however undetected in all analysed wines.

### Acknowledgement

The authors are grateful for the financial support provided by the Dirección Xeral de I+D – Xunta de Galicia (Project number 6RC2014/2016 *Grupos de Referencia Competitiva*), and European Regional Development Fund (FEDER), projects number GPC2014/035.

# THE USE OF INFRARED SPECTROSCOPY AND CHEMOMETRIC TOOLS FOR DETERMINATION OF FATTY ACIDS AND LIPID CLASSES IN SALMON OIL

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Near-infrared (NIR) and Mid-infrared (MIR) spectroscopies combined with chemometric tools have been evaluated for the determination of oleic, palmitic, linoleic, linolenic acids, fatty acid families as omega-3, omega-6, polyunsaturated fatty acids (PUFAs), monounsaturated fatty acids (MUFAs), saturated fatty acids (SFAs) as well as triglycerides, monoglycerides, ergosterol and free fatty acids.

54 different salmon oil extracts characterized by chromatographic techniques were employed. Partial Least Square (PLS) regression models were applied to correlate infrared spectra (NIR and MIR) with reference values on considering two independent calibration and validation sets of oil extract samples. Different spectral ranges and preprocessing techniques have been considered in order to obtain PLS models with a high predictive capability. Results obtained evidenced that both techniques can be used as direct and non-destructive method for a fast and cheap estimation of different lipids and fatty acids in salmon oil extracts, with relative root mean square error of prediction (RRMSEP) below 2% (see results in Table).

Table 1. Parameters of PLS-NIR and PLS-MIR models developed for the determination of fatty acids and lipid classes in salmon oil.

Analyte	LV	Calibration set				Validation set			
		RMSEC (% w/w)	R <sup>2</sup> <sub>cal</sub>	RMSECV (% w/w)	R <sup>2</sup> <sub>cv</sub>	RMSEP (% w/w)	R <sup>2</sup> <sub>pred</sub>	RRMSEP (%)	RPD
Near Infrared									
Omega-3	5	0.21	0.95	0.28	0.91	0.20	0.92	1.41	2.84
Omega-6	4	0.07	0.96	0.11	0.89	0.09	0.73	0.51	1.71
PUFAs	5	0.19	0.97	0.35	0.91	0.26	0.91	0.76	2.41
MUFAs	3	0.19	0.86	0.25	0.75	0.30	0.61	0.69	1.39
SFAs	7	0.08	0.74	0.13	0.40	0.12	0.63	0.62	1.62
Mid Infrared									
Omega-3	7	0.15	0.96	0.31	0.85	0.26	0.94	1.96	3.28
Omega-6	7	0.07	0.94	0.12	0.81	0.08	0.94	0.44	3.52
PUFAs	7	0.19	0.96	0.30	0.90	0.21	0.97	0.64	5.22
MUFAs	5	0.17	0.88	0.23	0.79	0.18	0.73	0.41	1.96
SFAs	9	0.07	0.86	0.16	0.44	0.09	0.67	0.45	1.79

LV: number of latent variables; RMSEC, RMECV, RMSEP: root mean square error of calibration, cross validation and prediction.

## Acknowledgements.

Authors acknowledge the financial support of Ministerio de Economía y Competitividad-Feder (Project CTQ 2014-52841-P) and Generalitat Valenciana (Project PROMETEO II/2014/077).



**ESTUDIO DE VIDA ÚTIL DE ACEITE DE OLIVA VIRGEN Y ADEREZADO MEDIANTE LUMINISCENCIA****E.J. Díaz-Montaña, D. Hernanz, M.T. Montaña, M.T. Morales**

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Actualmente, existe preocupación dentro del sector del aceite de oliva virgen por obtener métodos objetivos que permitan predecir, tanto la vida útil de los aceites, como su fecha de consumo preferente. Existen diversos compuestos presentes en el aceite de oliva virgen que poseen propiedades luminiscentes, entre los que se encuentran componentes menores como tocoferoles, compuestos fenólicos y pigmentos [1,2]. Durante la autooxidación del aceite se producen modificaciones en su composición y se originan compuestos químicos que modifican sus propiedades organolépticas y tienen efecto sobre su valor biológico [3]. Algunos de estos compuestos poseen propiedades luminiscentes cuya determinación puede ser útil para evaluar e incluso predecir la vida útil del aceite.

El objetivo de este trabajo es evaluar la estabilidad oxidativa del aceite de oliva virgen extra con y sin aderezo y establecer las diferencias que se producen entre ellos, utilizando técnicas espectroscópicas luminiscentes

El estudio se ha realizado con aceite de oliva virgen extra caracterizado desde un punto de vista químico y sensorial. Se hicieron tres lotes de muestras, sin aderezo, aderezado con romero y aderezado con albahaca, que se almacenaron en recipientes de vidrio transparente, en condiciones de temperatura ambiente y periodos de luz/oscuridad. Una vez al mes, durante un periodo de 12 meses, se adquirieron los espectros de emisión de las muestras a una  $\lambda_{ex} = 330$  nm y se evaluaron los parámetros de calidad físico-químicos.

Durante el periodo estudiado se observaron variaciones de intensidad en las bandas del espectro de emisión, debido a la degradación de tocoferoles, compuestos fenólicos y pigmentos, y a la producción de compuestos de oxidación. Estas variaciones fueron intensas durante los primeros meses de almacenamiento, produciéndose de forma gradual a partir del quinto mes, y observándose un cambio de pendiente en la evolución de las bandas relacionadas con la presencia de pigmentos. Se observaron diferencias en esta evolución entre las muestras con y sin aderezo, y entre las muestras con distinto tipo de aderezo.

Un análisis de varianzas puso de manifiesto la presencia de diferencias significativas ( $p < 0.05$ ) en diferentes zonas de los espectros que están relacionadas con los compuestos mencionados. Se obtuvo una correlación negativa entre la intensidad de algunas bandas del espectro, relacionadas con la presencia de tocoferoles, fenoles y pigmentos, y el tiempo de almacenamiento de las muestras, y una correlación positiva con la aparición de compuestos producidos por la oxidación de los aceites.

La aplicación del test de Brown-Forsythe y el análisis de componentes principales permitió seleccionar las  $\lambda_{em}$  más adecuadas para evaluar el tiempo de almacenamiento y establecer las diferencias cuali y cuantitativas que se producen por la presencia de plantas aromáticas, que retrasan y modifican el proceso de oxidación de las muestras respecto al aceite de oliva virgen control.

Por último, la aplicación de análisis discriminante permitió la discriminación de las muestras aderezadas de las no aderezadas y de las aderezadas entre ellas con altos porcentajes de clasificación y puso de manifiesto la influencia sobre los espectros del tiempo de almacenamiento de las muestras.

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**PLATAFORMA INDICADORA DE CALIDAD PARA DETERMINACIÓN DE  
FRESCURA EN ALIMENTOS.**

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Multisens, es un proyecto europeo que pretende revolucionar el mercado mediante el diseño de plataformas multisensoras que alerten del estado de frescura de alimentos, más concretamente, del estado de la carne empaquetada.

Tres sensores van a ser incorporados en las bandejas de carne de cerdo: CO<sub>2</sub>, NH<sub>3</sub> y H<sub>2</sub>S. Un cambio en el nivel de CO<sub>2</sub> en la atmósfera del envase es un claro indicador de que las bacterias están creciendo en el interior o de que el film protector que rodea al producto se ha roto. El NH<sub>3</sub> es un indicador de deterioro, ya que es un producto de degradación microbiana de proteínas. El H<sub>2</sub>S aparece por degradación de cisteína y es producido por el deterioro de la carne. Por ello, estos tres gases han sido seleccionados como gases diana para el desarrollo de sensores de frescura.

En primer lugar, se ha estudiado la correlación entre la frescura de la carne y la evolución de gases dentro de bandejas. Se ha estudiado el contenido microbiano de la carne en función del tiempo y a su vez, el contenido en gases dentro del envase. Así, se puede establecer el rango de cada gas a partir del cual la carne no es apta para consumo, en concreto, se ha seleccionado 10<sup>7</sup> cfu/g /mL como límite de frescura.

Los sensores a desarrollar para este proyecto se preparan en tintas de base agua, de modo que sean fácilmente implementables en la industria alimentaria mediante la impresión de los sensores en los plásticos que recubren las bandejas de carne.

Debido a la gran ventaja del uso de app en tablets y teléfonos móviles, pues se pueden utilizar sin necesidad de formación previa y desde cualquier lugar sin necesidad de estar en un laboratorio, se va a desarrollar una aplicación de modo que se pueda comprobar la frescura de los alimentos envasados incluso en el propio supermercado.

**Agradecimientos.** Este proyecto ha recibido fondos del programa H2020 de la Unión Europea grant agreement N° 706303 (Multisens) y del MINECO (CTQ2013-44545-R and CTQ2016-78754-C2-1-R).

## ARSENIC BIOACCESSIBILITY IN EDIBLE ALGAE

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Arsenic is a metalloid which can be found distributed through the environment. This element is a mobile pollutant and some of their species show health hazards. Inorganic species are more toxic than their organic analogues and these can be accumulated inside living organisms. Food contamination by arsenic is a problem in the world, especially in Asia due to the large number of cases of poisoning. Moreover, the European Food Safety Authority (EFSA) pointed out the need to produce speciation data, particularly inorganic arsenic data. Seaweeds are one of the food commodities that contain high levels of arsenic. For this reason, Hijiki seaweed (*Sargassum fusiforme*), Sea Spaghetti seaweed (*Himanthalia elongata*) and Wakame seaweed (*Undaria pinnatifida*) were selected for this study.

There are several approaches to determine the bioaccessibility as a previous step to determine the bioavailability, and all of them try to simulate the conditions presented in a human stomach. In-vitro methods provide effective approximations to in-vivo situations and offer the advantages of simplicity, rapidity, ease of control, low cost, high precision and good reproducibility.

The present work is aimed to assess total arsenic and arsenic species bioaccessibility in raw and cooked seaweed and to evaluate the potential health risk associated with their consumption. To achieve the conditions to simulate the digestion system a Physiologically Based Extraction Test (PBET) was applied. The research also includes the study of different cooking treatments. Raw and boiled samples were analysed to evaluate the bioaccessibility of arsenic.

Arsenic species were separated and measured by coupled techniques: liquid chromatography-inductively coupled plasma-mass spectrometry (HPLC-ICP-MS), and total extracted arsenic was quantified by ICP-MS. Total arsenic content of seaweed samples was determined by ICP-MS after a microwave digestion procedure. Furthermore, some seaweed certified reference materials were analysed to evaluate the accuracy of total arsenic and inorganic arsenic as well as to assure the quality of the results.

The total content of arsenic in samples was  $120,8 \pm 3,5$  mg kg<sup>-1</sup> for Hijiki seaweed,  $39,0 \pm 0,6$  mg kg<sup>-1</sup> for Sea Spaghetti seaweed and  $39,9 \pm 1,9$  mg kg<sup>-1</sup> for Wakame seaweed. The total content of arsenic decreased between 25 – 40% in boiled seaweed. The speciation showed that Hijiki seaweed mainly contains inorganic arsenic whereas Sea Spaghetti and Wakame seaweed mainly contains arsenosugars. The speciation of arsenic in boiled seaweeds indicated the degradation and transformation of some arsenic species.

The total arsenic bioaccessibility of raw seaweeds was between 75 - 90% approximately. Most bioaccessible fraction was extracted in the gastric step. Boiled seaweeds showed lower values ranging from 60 - 80% approximately. The results about inorganic arsenic exposition, only present in Hijiki seaweed, showed a bioaccessibility of 80% in raw seaweed and 60% in boiled seaweed, indicating the potential risk for health associated with their intake.

# FEASIBILITY OF A PORTABLE MINIATURIZED NEAR INFRARED SPECTROPHOTOMETER FOR DETERMINATION OF DRY MATTER CONTENT IN MEXICAN “HASS” AVOCADO

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Nowadays, the determination of harvest maturity (maximum potential quality) of avocado in Mexico is based on a rudimentary evaluation of indices such as mesocarp dry matter (DM), or moisture content (MC). The study of methods related to the ripening of avocado has been gaining interest and recently a review of destructive and non destructive methods for determining avocado maturity has been published [1] (Magwaza and Tesfay 2015). Near Infrared Spectroscopy (NIRS) has been used around the world as an alternative to traditional methods for predicting maturity parameters of fruits, including avocado, but the instruments are expensive and mainly for laboratory use.

In this work, a miniaturized Near Infrared Spectrophotometer: MicroNIR™, from Viavi Solutions (Milpitas, CA, USA), with two integrated vacuum tungsten lamps, and a 128-pixel InGaAs photodiode array as detector, weight < 60g and dimensions 45 × 42 mm (diameter × height), has been used to determine dry matter contents of Mexican avocados. For comparison, the same fruits were measured using a laboratory benchtop monochromator NIRSystems model 5000 spectrometer, coupled to an RCA (Rapid Content Analyzer) 6540A module (Foss NIRSystems, Silver Spring, MD, USA) with a quartz halogen lamp and PbS detector.

Mature “Hass” avocados were obtained from local supermarkets in Barcelona and were maintained at 20 °C (room temperature) before being scanned. The sampling point was selected following [2] “in the plane of the peduncle insertion point, but on the opposite side of the avocado at the equator”; where lowest variability on the DM content is observed. Approximately 10g of flesh avocado (every peel removed) was used to collect NIR spectra with both spectrometers. After first NIR spectra were recorded, the samples were subjected to different dehydration times, with the purpose of having a wide range of dry matter content in the calibration and prediction set.

The samples were split in two groups, a calibration set (156) and an external prediction set (87), and their spectral data were subjected to different pre-treatment methods to remove any irrelevant information which cannot be handled properly by the regression techniques, rectify multiplicative interferences of scattering and remove baseline shift. First and second derivative (D1 and D2), standard normal variate (SNV) and combinations SNV+D1, SNV+D2, were used. Calibration set was centered before PLSR, and the model was validated by cross-validation.

Using PLSR for MicroNIR 2D data, between 1100 and 1650 nm, the regression line of %DM calculated by NIRS versus the experimental values, for the prediction set, have a slope of 1.01 (s=0.03) and an intercept of 1.61 (s=1.88), R<sup>2</sup>=0.897. The Standard Error of Prediction (SEP) is 6.40. For benchtop Foss spectrometer, between 1100 and 2500 nm, the slope was 1.011 and the intercept 1.98. R<sup>2</sup>=0.923 and SEP is 5.17

These results open the possibility of carrying out rapid measurements directly in the field, by the easy portability of these mini instruments, and without a significant loss of the quality of the obtained data.

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## HACIA LA DETERMINACIÓN *IN SITU* DE AMINAS BIÓGENAS

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La industria alimentaria y los organismos de salud pública requieren métodos rápidos y económicos para el control de aminas biógenas (histamina, tiramina, cadaverina y putrescina fundamentalmente) en alimentos puesto que son un indicador de calidad y frescura de los mismos; además, a partir de ciertos niveles, su presencia está relacionada con la aparición de intoxicaciones e intolerancias [1].

En este sentido, nuestro grupo de investigación está desarrollando metodología analítica que pueda implementarse en tiras reactivas capaces de determinar de forma rápida e *in situ* la cantidad de diferentes aminas biógenas en alimentos o sus materias primas. Se basa en la utilización de reactivos orgánicos que cambian sus propiedades espectroscópicas al producirse una reacción enzimática en la que participan dichas aminas.

El esquema general es el que se muestra en la figura 1, en el que intervienen las enzimas Diamino Oxidasa (DAO) y Peroxidasa (HRP), y que se ha estudiado, con buenos resultados, con colorantes como ABTS (figura 2) y Amplex-Red, estableciendo las relaciones de concentración entre enzimas y colorante necesarias para obtener una señal estable en el tiempo, que dependa de la concentración de aminas.

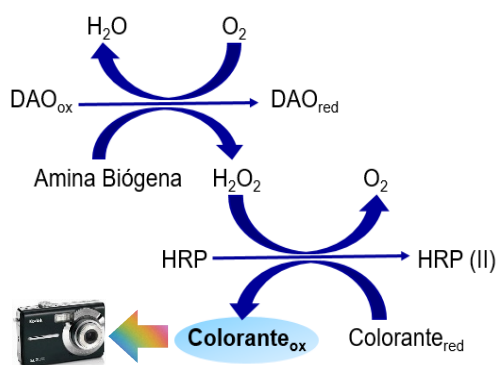


Figura 1: Esquema general del método colorimétrico enzimático para aminas biógenas.

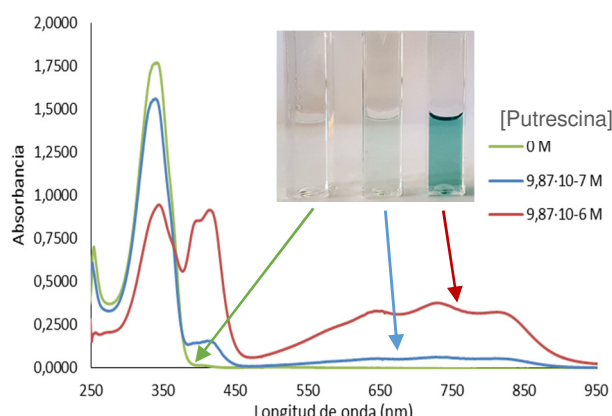


Figura 2: Señal del método colorimétrico utilizando ABTS como colorante.

Respecto a la reacción con ABTS se ha conseguido un método selectivo para putrescina y cadaverina, en el que se han realizado los primeros estudios sobre tiras reactivas (utilizando como soporte kits comerciales para  $H_2O_2$ ) que, mediante detección visual, responden a la presencia de niveles de estas aminas superiores a los permitidos legalmente.

En el caso del Amplex-Red, se ha conseguido obtener señal adecuada para putrescina, cadaverina e histamina, ampliando así la aplicabilidad del método. Además el producto de este colorante es fluorescente, mostrando también una señal dependiente de la concentración de aminas que pueda cuantificarse utilizando equipos portátiles.

En todos los casos, se están adaptando los métodos colorimétricos para la detección y cuantificación a través de las cámaras de dispositivos móviles.

Este trabajo ha sido realizado con cargo al Proyecto CTQ2016-76846R (MINECO) y las ayudas a grupos de investigación DGA-FEDER. Jesús Navarro agradece a la DGA por la concesión del contrato predoctoral en formación.

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## MERCURY ANALYSIS IN FOODS

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Nature offers us vegetables to our diet, there are many myths and legends that loom over the vegetables. In particular, there is a widespread misconception that popularly argues that legumes are fattening, even though they are found at the base of the Mediterranean diet.

The purpose of this project was to determine the amount of mercury in a total of twenty different types of legumes, comparing in turn, different varieties of them and different origin. To do this, a direct mercury analyzer (DMA-80) which does not require treatment of the sample was used. Due to the need to preconcentrate the mercury by amalgamation, a total of nine measurements per sample were conducted. All the samples show mercury results below  $1 \text{ ng g}^{-1}$

Optimization of working procedure for DMA-80, modifying some variables such as sample mass, drying and pyrolysis temperature and mineralization of the sample, was studied.

The analytical characteristics of the method were established, detection limits of  $0.021 \text{ ng g}^{-1}$ , quantification limit of  $0.071 \text{ ng g}^{-1}$ , precision and accuracy of the equipment by certified fly ash and rice flour samples.

A comparison was made between the origin of the samples and between varieties of them, with no significant differences.

The authors acknowledge the financial support of the Generalitat Valenciana Project PROMETEO II 2014/077

**RELIABLE METABOLIC PROFILING APPROACH TO DETERMINE PHENOLIC COMPOUNDS FROM VIRGIN OLIVE OIL: DIRECT INJECTION OF THE SAMPLES INTO THE LC-MS SYSTEM**

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The characterization of what we eat taking advantage of sophisticated analytical tools is a subject of major interest nowadays for diverse reasons, such as authentication purposes, understanding its healthy properties, demonstrating typicity of a product, etc. A LC-MS method involving direct injection of extra-virgin olive oil (EVOO) -after a simple dilution- for determining its phenolic compounds has been developed. During the optimization we had (among other things) to: select the most appropriate solvent for the sample dilution; choose the optimum oil/solvent ratio; establish a column cleaning strategy which could guarantee the proper performance of the column; and define the maximum number of injections (before every cleaning cycle). Afterwards, the analytical parameters of the method were evaluated, establishing LOD (from 3.3 to 31.6 µg/L) and LOQ, precision (RSD values for inter-day repeatability were found between 3.49 and 6.12%), and trueness (within the range 89.9-102.3% for 1.0 mg/L) and checking possible matrix effect (which was no significant). Three kinds of calibration were used: external standard, standard addition and calibration in a phenols-free matrix, which was subsequently applied to quantify the phenolic compounds in 16 EVOOs (from 6 cultivars). A total of 21 compounds were determined without the need of using any extraction protocol.

The possibility of implementing direct injection of olive oil into the LC could be one of the greatest advantages of this method, since it could prevent partial and selective recovery of some phenolic compounds after the extraction, and their possible partial oxidation or the creation of artificial isomers during the sample preparation.

## PRECONCENTRATION OF HALOANISLES BY CLOUD POINT EXTRACTION FOR THE ANALYSIS OF ALCOHOLIC BEVERAGES BY GC-MS

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Cloud point extraction (CPE) is a sample preparation technique based on the formation of micelles in a solution of non-ionic surfactants heated above their cloud point temperature. The main advantage of this technique respect to other liquid-liquid microextraction (LLME) procedures is that the use of toxic organic solvents is avoided, which is in agreement with the Green Analytical Chemistry guidelines [1].

The direct introduction of the coacervate into a gas chromatography (GC) system is impossible by means of conventional injectors because of the low volatility of the coacervates. Direct microvial insert thermal desorption [2] is here presented as an alternative to avoid additional steps after CPE, such as back-extraction of the analytes into an organic solvent compatible with GC [3] or derivatization of the surfactant contained in the coacervate [4]. Direct microvial insert thermal desorption approach is based on the use of a commercial thermal desorption unit (TDU) as interface to transfer to the GC the preconcentrated analytes from the surfactant rich phase. For this, glass inserts containing up to 150  $\mu\text{L}$  of extractant phase are placed in the thermal desorption tube, and next the whole assembly submitted to a programme temperature into the TDU. A carrier gas propels the analytes to a programmed temperature vaporizator (PTV) injector, where they are focused before entering the chromatographic column. Next, the PTV is heated, and the retained compounds enter the GC system. In this way, the surfactant hardly reaches the GC system, and, even if some vapours are dragged by the gas flow, they are retained in the disposable PTV liner.

In this communication GC analysis of the CPE extracts using microvial insert thermal desorption is proposed for the determination of four haloanisoles (2,4,6-trichloroanisole (2,4,6-TCA), 2,4,6-tribromoanisole (2,4,6-TBA), 2,3,4,6-tetrachloroanisole (2,3,4,6-TeCA) and pentachloroanisole (PCA)) related with cork taint defects in wines, in different types of alcoholic beverages. The haloanisoles were extracted from the matrix samples by CPE using Triton X-114 heated to 75  $^{\circ}\text{C}$ , the surfactant rich phase was separated by centrifugation and 20  $\mu\text{L}$  of the CPE obtained extract was submitted to GC with mass spectrometry (MS) analysis. The parameters affecting the CPE (nature and concentration of the surfactant, ionic strength and pH of the donor phase as well as temperature and time for the heating step) and the microvial insert thermal desorption (vaporization time and temperature in the PTV injector, carrier gas flow-rate, focusing temperature, filling material in the PTV liner as well as time and temperature in the CIS) steps were optimized.

Different wood-aged alcoholic beverages, including white and red wine, beer and whisky were analysed under the finally selected conditions. For CPE, volumes of 5 mL of beverage samples were submitted analysis, whereas a 1:4 water:sample dilution was previously applied for whiskies. Quantification was carried out by matrix-matched calibration using 5-bromo-2-chloroanisole as internal standard. Detection limits ranged between 12.9 and 20.8  $\text{ng L}^{-1}$ , depending on the compound, for beer and wine samples, whereas for whiskies values in the 46.3-48  $\text{ng L}^{-1}$  were obtained, since these samples were diluted for analysis. Recoveries for alcoholic beverages were in the 89-111% range, depending on the haloanisole and the sample.

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The authors acknowledge the financial support of the Comunidad Autónoma de la Región de Murcia (CARM, Fundación Séneca, Project 19888/GERM/15), the Spanish MINECO (Project CTQ2015-68049-R) and the European Commission (FEDER/ERDF).



## USE OF SMARTPHONE CAMERA TO QUANTIFY CALCIUM IN FRUITS

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Plant often appears to be nutrient deficient in highly exploited crops due to the intensive culture rate employed to achieve high benefits. This nutrient deficiency will lead to greatly cut down the quality of the plants and consequently a decrease in the fruit production. For this reason, cheap, fast and efficient methodologies to determine the plant problems with enough time to correct the deficiency is an important issue nowadays. Especially, since agriculture is destined to solve the problems of lack of food that are coming due to the population exponential increase.

Usually, the analysis methods to determine nutrient deficiency in plants are made by expensive and slow techniques as atomic absorption or emission spectroscopy, inductively coupled plasma and others. These methodologies have several problems as their elevated cost (also for maintenance) and time to realize the analysis. Other problems associated to the analytical techniques is the need of a good sampling and sometimes-tedious sample treatments. These led to imprecise results, not only due to the techniques or to the sampling time, but also due to the differences between plants, because, as people, not all the trees in crops are going to have the same problems.

Therefore, the development of new fast, cheap and easy techniques to analyze crops is necessary. In this work, the first approach to develop a new methodology to determine calcium, a typical nutrient deficiency problem, in cherries by the employment of mobile phone camera is presented. With this aim, the fruits were photographed with an android mobile phone and the corresponding photographs were treated in OriginLab® software to convert to black and white and to obtain the resultant numerical matrix, where some lines in different points were selected and transported to the IBM SPSS® software to make a polynomial least squares (PLS) analysis. The method is able to quantify the calcium content in the skin of cherries among other parameters analyzed (as potassium, humidity, etc.) in new samples by interpolating the adequate variables in the model.

This new methodology led to in situ, cheap, easy and fast determination of nutrients in plants. In addition, the developed method allows the detection of problem in specific trees or plants, which could be translated in save cost expenses in correctors inasmuch as only specific plants/trees should be treated.

**VOLTAMMETRIC ANALYSIS OF ARCHAEOLOGICAL GOLDEN THREADS**

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**Abstract**

Solid state electrochemical techniques are applied to the analysis of golden threads from different archaeological sites (l'Almoyna and Cisneros street) in Valencia (Spain). Upon attachment of nanosamples from thread fragments 1 mm sized to graphite electrodes immersed into 0.10 M HCl aqueous solution, characteristic features of gold oxidation were detected. Voltammetric data, supported by SEM/EDX analysis of cross-sections permitted to determine the presence of Ag and, in few cases of Hg, thus denoting the use of amalgamation as a fabrication procedure. The study of the current ratios for gold oxidation and oxygen evolution reaction provided grouping of the samples into different historical periods (Roman, Islamic and 18<sup>th</sup> century, respectively).

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A CASE OF ELECTROCHEMICAL DETECTION OF GOLD FORGERIES

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**Abstract**

The detection of forgeries is an important analytical target for museums and collections. Among other techniques, solid state voltammetry can be used for this purpose by virtue of its inherently high sensitivity and the minimally invasive character (samples in the nanogram order can be used) of the technique [1]. The application of the same for detecting gold forgeries is illustrated here by a *torqués*, presumed to be made of gold, pertaining to the reservoir of the Museum of Borriana (Spain). The object was found in the archaeological site of *La Muntanyeta* (Betxí, Spain) in 1991 and deposited in the Museum of Borriana with uncertain attribution to pre-Iberian times. Voltammetric data for nanosamples of the *torqués* attached to graphite electrodes in contact with 0.10 M HCl aqueous solution revealed the absence of the typical signatures for gold oxidation at potentials around +1.0 V vs. Ag/AgCl [2,3] as well as signals attributable to silver often accompanying gold in golden artifacts. On the contrary, voltammetric data indicated the presence of copper as the main component of the sample, judged to be made of brass upon comparison with pertinent reference materials.

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**CHEMICAL CHARACTERISATION OF CHERTS FROM THE VALLEY OF SERPIS RIVER  
(ALCOY, ALICANTE) FOR ARCHAEOLOGICAL PURPOSE**

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Mobility range and territorial control are central questions for Archaeology in the study of human groups' life during Prehistoric Ages. A key point to get to grips with this set of problems is to understand the dynamics of supply of natural resources such as food and raw materials. Thus, the identification of the outcrops of chert and their characterisation is essential, due to the use of this particular rock as raw material for the production of several different tools. Since the naked-eye description of stone characters (colour, translucency, presence of carbonatation or patina, etc.) often lacks to identify different outcrops and to determine the provenance of a sample, in the last decades, scientists have tried to develop methods to improve the characterisation of this rock from the chemical, mineralogical and petrographic point of view [1,2].

This contribution shows the study of some chert varieties which were widely used since the Paleolithic by the inhabitants of the valley of Serpis river [3], in the southern part of the Valencian Community. Forty-three samples of *Serrat*, *Mariola* and *Serreta* chert were collected from different kinds of outcrops: from the wall rock, and from fluvial and colluvial deposits. The cortex or crust and the nucleus of each sample were mechanically separated and individually analysed to control the variability caused by the amount of cortex and consequently to develop a methodological approach that permits to identify different chert sources in a restricted area. For this purpose, X-ray fluorescence and Inductively coupled plasma mass spectrometry analyses have been carried out to determine major elements, trace elements and rare earth elements [4] of cherts affected by different depositional and post-depositional conditions.

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**CHEMICAL CHARACTERISATION OF HISTORIC MORTARS TO EVALUATE  
DECAY AND CONSTRUCTION PHASES**

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The chemical characterization of ancient mortars allowed the researchers to answer relevant questions about production technologies, raw materials supply, construction phases and state of decay.

In this work one hundred and sixteen samples were collected from different structures during two archaeological excavations carried out in Sagunto's city centre (Valencia, Spain). The studied area has been interested by several continuous phases of occupation since the Iberian Epoch (5<sup>th</sup> century BC) to the present times [1,2]. The samples were analysed employing X-ray fluorescence and Inductively coupled plasma mass spectrometry to determine major and trace elements. The obtained data was statistically processed with Sagunto's Castle mortar results [3], allowing us to identify the construction phases of most of the wall structures, confirming the particular effectiveness of Rare Earth Elements analysis to distinguish mortars from different periods. In conclusion, according to this data, the state of conservation of the different mortars has been evaluated.

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## ANÁLISIS QUÍMICOS NO DESTRUCTIVOS SOBRE CINCO MANGOS DE MARFIL DE ÉPOCA IBÉRICA

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Presentamos los análisis químicos realizados sobre un conjunto de cinco mangos de marfil época ibérica (s. VI – s. I a. C.). Son objetos con características técnicas, formales y decorativas muy similares entre sí, lo que permite plantear una relación entre ellos en su proceso de producción. Sin embargo, fueron recuperados en cuatro yacimientos diferentes del área ibérica: los poblados de Turó de Montgròs (El Brull, Barcelona) y La Serreta (Alcoi, Alicante) y las necrópolis de El Cigarralejo (Mula, Murcia) y Coimbra del Barranco Ancho (Jumilla, Murcia), en esta última, se recuperaron dos de ellos. Todas las piezas, por sus contextos arqueológicos se datan entre el s. IV y principios del s. II a. C.

Los análisis se llevaron a cabo con el objetivo de identificar la naturaleza y procedencia de las incrustaciones de carácter decorativo y la sustancia adherente todavía presentes en estos mangos. Si bien, en algunos de ellos, debido a su deteriorado estado de conservación, únicamente quedaban las improntas de las incrustaciones y no había restos aparentes de la sustancia de tono gris-negro que, presumiblemente, serviría para adherir las incrustaciones. Las piezas mejor conservadas son las recuperadas en lugares de hábitat.

Los estudios se han realizado mediante técnicas no destructivas que no comprometiesen la integridad de las piezas. Así, se llevaron a cabo análisis por Fluorescencia de Rayos X (XRF), y por Espectrofotometría de Infrarrojo Cercano por Transformada de Fourier (FT-NIR). Todo ello se complementó mediante la revisión de los mangos con un microscopio electrónico de barrido equipado con un Sistema de Rayos X de Energía Dispersiva (SEM-EDAX-Sapphire), un microscopio óptico SMZ (NIKON) y un microscopio digital Dino-lite mod. AM7115MZZT EDGE de 10x a 200x con una luz incidente por medio de un iluminador de fibra óptica y dotado de un software con funciones de medición integrales, para obtener imágenes de alta precisión.

El análisis de XRF ha permitido identificar como estaño la sustancia empleada para adherir las incrustaciones decorativas en la pieza, empleando una técnica de tipo soldadura blanda. El FT-NIR, por su parte, ha revelado que dichas incrustaciones fueron realizadas sobre resinas fósiles, muy probablemente ámbar. Estos resultados resultan totalmente novedosos dentro del mundo artesanal de época ibérica y, por tanto, de gran interés, evidenciando el valor de estas piezas en las que materias primas de presencia escasa en el mundo ibérico como son el marfil y el ámbar aparecen combinadas; así como el uso del estaño a modo de soldadura blanda nos revela procesos de manufactura no atestiguados hasta el momento en las industrias sobre materias duras de origen animal de la Edad del Hierro en la Península Ibérica.

Cabe destacar que la realización de análisis químicos sobre piezas arqueológicas aporta datos específicos, prácticamente imposibles de obtener en un estudio macro o microscópico de los artefactos. Por ello, desde el proyecto proyecto *“Madera, hueso, marfil, asta, concha ¿Artesanías marginales o marginadas?”* (HAR2013-45770-P y ACOMP/2015/256) (financiado por el Ministerio de Economía y Competitividad y la Generalitat Valenciana) dirigido por la Dra. Consuelo Mata, se ha apostado por desarrollar estudios interdisciplinares, que impliquen el contacto y la comunicación entre distintos especialistas con la finalidad de conseguir resultados más sólidos y transversales.

**A PAPER-BASED ANALYTICAL DEVICE FOR SCANOMETRIC DETECTION OF SALIVARY THIOCYANATE****F. Pena-Pereira, I. de la Calle, V. Romero, I. Lavilla, C. Bendicho**

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The development and application of paper-based analytical devices (PADs) with instrumental-free analytical systems is receiving increasing attention in recent years [1]. The present work reports on the development of a non-instrumental sensing approach for the detection of salivary thiocyanate, an important biomarker for tobacco smoke exposure [2]. The proposed methodology is based on the implementation of the well-known spectrophotometric method involving iron(III)-thiocyanate complex formation under acidic conditions on a paper substrate [3,4]. PADs were prepared by adapting, with slight modifications, the fabrication process reported by Nie *et al.* [5]. In brief, detection areas were plotted on both sides of a paper substrate by using a permanent marker. After evaporation of the solvent ink, hydrophobic barriers with the required width were thus obtained, and the detection zone was modified with a microvolume of Fe(III) reagent solution. After exposure to blanks, standards or samples, the analytical response (mean color intensity) was obtained by digitizing the detection zone of the PAD by means of a scanner, and subsequently processing the obtained images with ImageJ, a free image processing and analysis software. Different experimental parameters that affect both the preparation of PADs and the performance of the scanometric method were assessed, namely color mode detection conditions, paper substrate type, average width of hydrophobic barriers required to obtain functional test zones, detection zone dimensions, detection zone composition and analysis time. Furthermore, the stability of the prepared PADs was assessed at different storage temperatures. Under optimal conditions, the limits of detection and quantification, calculated at a signal-to-noise ratio of 3 and 10, were found to be 0.06 and 0.21 mM, respectively. The linear dynamic range was established in the range 0.25-20 mM, with a regression coefficient of 0.9995. The precision of the method, evaluated as repeatability, was studied at a 1 mM concentration level by performing twenty five consecutive measurements, and found to be 3.0% when expressed as relative standard deviation. In comparison with alternative methods reported for determination of thiocyanate in saliva, the proposed method involves cheap and easily available materials and a remarkably low consumption of chemicals, thus resulting in a highly reduced cost per sample for detection of salivary thiocyanate. The method was finally applied to the analysis of saliva samples of twelve volunteers, with average thiocyanate contents in the ranges 0.28-0.87 mM and 0.78-4.28 mM for smokers and non-smokers, respectively. Recovery studies were also performed at two concentration levels to evaluate for potential matrix effects. Recovery values were in the range of 96-104%. Furthermore, the accuracy of the method was evaluated by statistically comparing the obtained results with those obtained by means of the reference spectrophotometric method [3]. A good agreement between the results provided by both methods was observed since  $t_{exp} < t_{crit}$  (paired t test,  $\alpha$  0.05, 2 tails).

**Acknowledgements:** Financial support from the Spanish Ministry of Economy and Competitiveness (Project CTQ2015-68146-P) (MINECO/FEDER) is gratefully acknowledged.

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# NOVEL INSIGHTS INTO THE POTENTIAL OF SELENIUM NANOPARTICLES AS A CHEMOTHERAPEUTIC AGENT: BIOMOLECULAR MECHANISMS, *IN VIVO* STUDIES AND CELL-SPECIFIC TARGETING

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Selenium is an essential trace element that plays a vital role in human health. Many studies have suggested the utility of Se as a potential anticancer element due to its antioxidant properties [1]. This has flared widespread interest in selecting the most suitable Se species, in understanding the therapeutical biomolecular mechanisms and in optimizing the way Se can be delivered to targeted cancer cells or combined with synergic therapeutic cargoes.

In a previous study, we described the differential effect of several inorganic and organic selenospecies on the growth and proliferation of hepatocarcinoma cells (HepG2) [2]. Among the different tested species, Se nanoparticles (SeNPs) turned out to be particularly promising by inducing significant changes in the cell cycle pattern of cancer cells without resulting highly toxic for healthy cells.

In this study, we performed a quantitative proteomic (SILAC) experiment directed toward identifying differentially expressed nuclear proteins in HepG2 cells upon SeNPs exposure. Cells exposed to SeNPs were grown in a culture media containing “heavy” isotopic amino acids (<sup>13</sup>C-Arg and <sup>13</sup>C-Lys) while control cells were grown in “light media” (<sup>12</sup>C-Arg and <sup>12</sup>C-Lys). After complete labeling, nuclear proteins from both conditions were isolated, separated by SDS-PAGE and trypsin digested prior mass spectrometry analysis. We identified and quantified a number of altered proteins that were further validated by qPCR. These results have allowed us for a better understanding of the biomolecular mechanisms underlying the effects of SeNPs on cancer cells.

Since all the *in vitro* experiments performed pointed at the potential of SeNPs to decrease the proliferation of cancer cells, we designed and carried out the following *in vivo* experiment: mouse melanoma cells (B16-F10) were used to induced a subdermal tumor in C57BL6 mice. 0.5 mm<sup>2</sup> tumors were treated with SeNPs intra-dermically. Tumor growth was monitored during 10 days in order to evaluate the potential of SeNPs to inhibit proliferation.

For clinical administration of nanoparticles, an effective targeting is vital for preventing harmful side effects and for improving the therapeutic profit [3]. Thus, we designed a hybrid nanosystem consisting of SeNPs dispersed on mesoporous silica nanoparticles (MSNs) functionalized with a ligand for cancer cell-specific targeting. We optimized the synthesis of the nanosystem and characterized the final nanomaterial by different techniques (TEM, EDX, DLS, etc.). Finally, we tested the efficiency of the targeting in several cells lines by different bioanalytical approaches.

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# COVALENT ATTACHMENT OF BIOTINYLATED MOLECULAR BEACONS VIA THIOL-ENE COUPLING. A STUDY ON CONFORMATIONAL CHANGES UPON HYBRIDIZATION AND STREPTAVIDIN BINDING

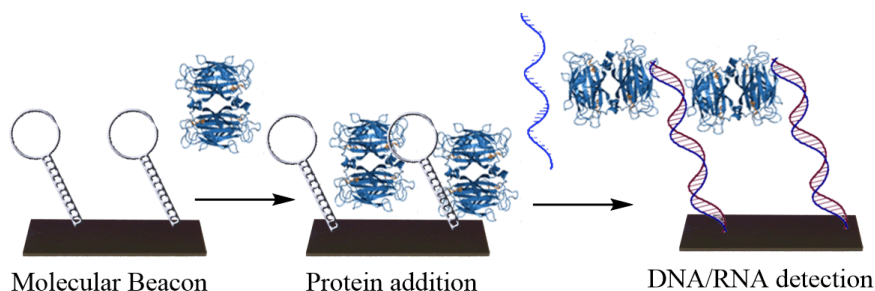
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Molecular beacons (MBs) are a class of DNA probes which are single stranded oligonucleotides that possess a stem-loop structure. They are an excellent example of biomolecular analysis probe that appears to be very promising for genomic studies.<sup>1,2</sup> However, few studies exploit the MB hairpin conformation on surfaces for the detection of DNA by hybridization measuring fluorescence.<sup>3,4</sup>

Immobilization of MBs was achieved on Silicon on Insulator (SOI) surfaces with only 30 s photo-immobilization, producing high immobilization densities ( $14.5 \pm 0.5$  pmol·cm<sup>-2</sup>). The anchored MBs were able to hybridize with the complementary target with high selectivity and sensitivity in microarray format (LOD =  $9.9 \pm 1.1$  nM).



MBs with a biotin moiety in one side of the stem were able to incorporate a streptavidin protein in a straightforward manner by strong biotin-streptavidin interaction. This process opens the possibility to incorporate a wide range of streptavidin-linked reporters that are currently commercially available. MBs with the attached streptavidin were able to hybridize with the complementary target in the presence of the protein.

In order to better understand the conformational changes that occur to immobilized MBs upon hybridization, the process was studied by using dual polarization interferometry (DPI). A model system was developed that matches thickness, mass, and density parameters. The results experimentally demonstrates for the first time that hybridization promotes the displacement of a protein away from the surface. This finding can be directly applied to novel label-free biosensors based on MB technology as virtually any reporter can be incorporated to MBs without significantly affecting the hybridization process.

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**HIGHLY SPECIFIC LABEL-FREE BIORECOGNITION ASSAYS BY SURFACE-ENHANCED RAMAN SCATTERING ON SILVERED POLYCARBONATE STRUCTURES****M. Avella-Oliver, G. Sancho-Fornes, N. M. do Nascimento, R. Puchades, A. Maquieira**

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Biomacromolecular probes (DNA, antibodies, enzymes, etc.) provide biorecognition assays with exquisite selectivity. However, even higher selectivity is very often required to successfully perform direct analysis in complex matrixes, such as blood or urine. Surface-enhanced Raman scattering (SERS) involves great potential to offer solutions in these regards [1]. Unlike other phenomena exploited for biosensing (fluorescence, SPR, interferometry, etc.), the distinctive strength of SERS lies in providing characteristic fingerprint spectra of the targets under study. Herein we aim to join biorecognition assays with SERS to conceive sensitive and extremely selective label-free bioanalytical systems. First, the development of mass-produced low-cost SERS substrates was addressed by coating with silver grooved polycarbonate structures created from standard compact disks. These substrates showed significant Raman enhancement, tunable plasmonic behavior, and are about two orders of magnitude cheaper than their commercially-available counterparts. Then, we arranged a simple detection scheme to perform label-free immunoassays for direct SERS analysis of low-molecular weight organic compounds, tailored to obtain the characteristic fingerprint spectra of the analytes. For that, specific antibodies have been microarrayed on the SERS-active silvered surfaces, and key aspects of this biorecognition (immobilization, antibodies fragmentation, etc.) have been assessed in order to increase the sensitivity of the assay. This approach also enhances the multiplexing capabilities, since different biological probes can be immobilized in a single spot in order to simultaneously analyze many analytes with different Raman spectra.

Acknowledgement: this work was supported by the Spanish Ministry of Economy and Competitiveness (CTQ2013-45875-R), FEDER, and the Generalitat Valenciana (PROMETEO II/2014/040).

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**A PRACTICAL METHOD TO INCREASE SENSITIVITY IN OPTICAL BIOSENSING**

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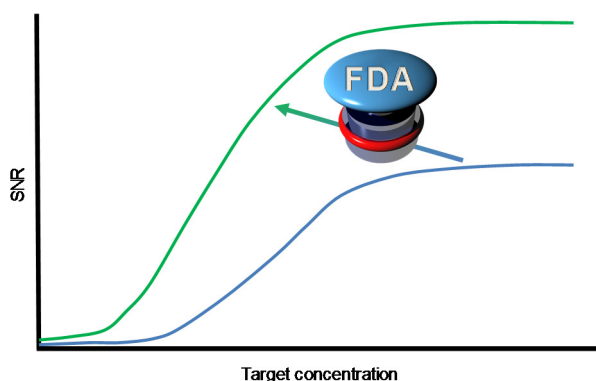
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Analytical signals in optical biosensing involves, together with the magnitude of interest, important contributions from external physicochemical sources (noise) that cause significant sensitivity losses. Among the different strategies carried out to solve this problem [1], digital filtering is the most commonly employed.

Here, we present frequency-domain analysis (FDA) as a novel and practical method to efficiently identify the analytical signal of interest and discriminating every noise contribution and therefore enhance sensitivity in optical biosensing. This approach involves biological probes patterned as straight and equidistant strips on solid substrates, in order to generate periodic signals when they are analysed by an optical scanner. FDA converts these periodic time-domain signals into their frequency-domain counterpart, using the Fourier transform. As a result, their signal contributions are unified in particular peaks, whereas undesired noise is spread along the spectra.

In this study, a disc-based immunosensor for specific IgGs determination was used as representative model system to prove the biosensing performance of FDA. The experimental results show detection and quantification limits improvement of more than 2 and 3 orders of magnitude, respectively.

Acknowledgement: this work was supported by the Spanish Ministry of Economy and Competitiveness (CTQ2013-45875-R), FEDER, and the Generalitat Valenciana (PROMETEO II/2014/040).



**Fig. 1 Scheme of the sensitivity improvement obtained by FDA..**

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**DETERMINATION OF KETONES AND ETHYL ACETATE BY HEADSPACE- PROGRAMMED TEMPERATURE VAPORIZER- GAS CHROMATOGRAPHY-MASS SPECTROMETRY. A PRELIMINARY STUDY FOR THE DISCRIMINATION OF PATIENTS WITH LUNG CANCER AND CONTROLS**

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In this work, ten possible volatile biomarkers of lung cancer (acetone, 2-butanone, ethyl acetate, 2-pentanone, 4-methyl-2-pentanone, 2-hexanone, 3-heptanone, 2-heptanone, 3-octanone and 2-nonanone) have been analyzed to evaluate their concentration differences in urine samples from lung cancer patients ( $n = 12$ ) and healthy controls ( $n = 12$ ). The volatile compounds were generated with a headspace autosampler and analyzed with a gas chromatograph equipped with a programmed temperature vaporizer and mass spectrometry detection (HS-PTV-GC-MS). With the aim of evaluating the aforementioned differences, a Mann-Whitney U-test and box-plots were obtained. Very good discrimination between cancer and control groups was achieved for three (ethyl acetate, 3-heptanone and 3-octanone) of the ten analytes studied. With a view to assigning samples to the healthy or patient group, the Wilcoxon signed-rank test has been used. In spite of the small number of urine samples assayed, the results may suggest that the studied compounds could be considered as useful tools for discerning samples and it could be taken as a complementary test in diagnosis.

## DETECCIÓN DE MUTACIONES EN ONCOGENES EN EL TRATAMIENTO DIRIGIDO AL CÁNCER COLORRECTAL

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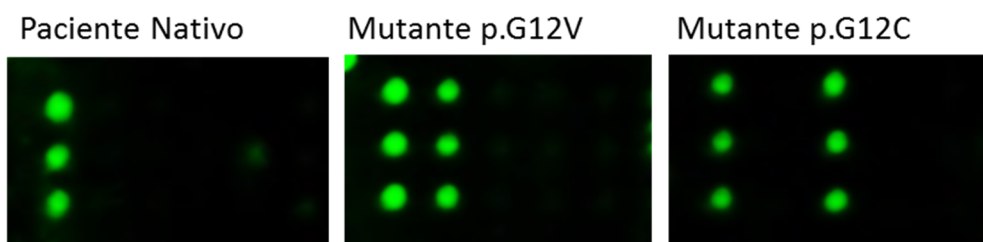
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La presencia de mutaciones somáticas en ciertos oncogenes confiere resistencia a los tratamientos con anticuerpos monoclonales [1]. Por ello, las guías oncológicas indican que es necesario descartar la presencia de la mutación para comenzar la administración de los fármacos. Sin embargo, no todos los centros hospitalarios disponen de las tecnologías necesarias. Esto conlleva retrasos en el inicio de la administración del fármaco (o su alternativa) con el consecuente empeoramiento del estado del paciente. Además, en los últimos años se está investigando cómo identificar las mutaciones presentes para una mejor clasificación del tumor [2].

En este estudio, se ha desarrollado un método para la detección e identificación de mutaciones presentes en biopsias de pacientes con cáncer colorrectal. Dada la baja proporción de las variantes mutantes respecto a las variantes nativas, se han estudiado técnicas de enriquecimiento [3]. Se han puesto a punto amplificaciones bloqueadas basadas en la adición a la mezcla de reacción de un oligonucleótido que reconoce a la variante nativa y limita la elongación de los cebadores por acción de la polimerasa. La selección de los oligonucleótidos de bloqueo se ha realizado en base a estudios termodinámicos de la estabilidad de los complejos que forman con el ADN molde. Para confirmar los productos formados, se han estudiado diferentes estrategias de biosensado óptico.

El reto de la identificación de la mutación se ha abordado mediante un ensayo de micromatrices [4,5]. Para ello, las sondas específicas para cada mutación han sido ancladas al chip fotoquímicamente activado. Tras el ensayo de hibridación, se ha realizado la detección fluorescente directa y la detección colorimétrica tras un revelado inmunoenzimático. Las imágenes resultantes han mostrado un perfil de respuesta diferencial en función del grupo poblacional (ej. gen *KRAS* codón 12).



Las prestaciones analíticas alcanzadas muestran el potencial del método desarrollado para el análisis de cultivos celulares y tejidos biopsiados. Con los resultados alcanzados en el presente estudio, se contribuirá a aumentar el número de pacientes que puedan beneficiarse de una terapia personalizada.

**Agradecimientos.** Proyecto MINECO RTC-2015-3625-1

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## SELECCIÓN DE DETERMINANTES ANTIGÉNICOS PARA LA DETECCIÓN DE ALERGIAS A ANTIBIÓTICOS $\beta$ -LACTÁMICOS

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Los antibióticos  $\beta$ -lactámicos (BLC) son los fármacos más utilizados y prescritos en la UE para combatir infecciones microbianas sistémicas en humanos, contribuyendo al 50% o más del consumo total de antibacterianos. La hipersensibilidad a estos medicamentos comprende más del 10% de las reacciones alérgicas adversas agudas.

Las pruebas inmunoquímicas in vitro de diagnóstico de alergia a antibióticos  $\beta$ -lactámicos se basan en la determinación de la concentración de IgEs específicas en suero sanguíneo. Actualmente, estas pruebas se limitan a la detección del efecto alergénico de un número reducido (3-5) de  $\beta$ -lactámicos y muestran una tasa alta de falsos diagnósticos, lo que provoca hospitalizaciones innecesarias y el uso de fármacos alternativos más caros, que pueden ser menos efectivos y peor tolerados.

La búsqueda de nuevos determinantes alergénicos es clave para el desarrollo de mejores pruebas de diagnóstico in vitro, de alta sensibilidad y selectividad. Esto comprende, en primer lugar, la selección y cribado de antígenos que cubran un amplio espectro de epítomos de las IgEs diana.

Este trabajo presenta resultados del estudio de estrategias químicas para el desarrollo de determinantes antigénicos principales y minoritarios para las familias de las penicilinas (penicilina G y V, amoxicilina, ampicilina, piperacilina, y carbacilina), cefalosporinas (ceftriaxona y cefaclor), carbapenemas (aztreonam) y monobactámicos (meropenem), con el fin de aumentar el espectro de epítomos de IgEs. Por un lado, dichas estrategias se centran en la apertura del anillo  $\beta$ -lactámico mediante un ataque nucleofílico, obteniendo los determinantes principales (-oyl). Por otro lado, la obtención de determinantes minoritarios (-ayl), se abordó mediante reacciones químicas de unión de grupos funcionales, como: la unión a través del ácido carboxílico del anillo de tiazolidina, en el caso de las penicilinas, o del anillo de dihidrotiazina, en el caso de las cefalosporinas; y a través del grupo amino de la cadena lateral. La selección se realizó en base a criterios de selectividad y sensibilidad, empleando un inmunoensayo heterogéneo directo en formato de micromatriz con detección colorimétrica para el análisis de sueros humanos artificiales preparados *ad hoc*.

Todos los determinantes antigénicos, independientemente de la ruta empleada, permitieron el análisis de muestras de sueros artificiales en el intervalo de concentraciones de IgEs que corresponden a los ensayos in vitro de Clase 1 (0.35-0.7 KU/L) según la clasificación RAST. Respecto a la selectividad, todos los determinantes antigénicos preparados fueron específicos para cada familia de BLC estudiada.

### Agradecimientos:

Este trabajo ha sido financiado por el programa H2020 (proyecto COBIOPHAD, *grant agreement* No. 688448), el Ministerio de Economía, Industria y Competitividad (referencia CTQ-BQU2013-45875-R), FEDER y la Generalitat Valenciana (Referencia Prometeo II/2014/040).

E.P.M agradece la ayuda predoctoral F.P.U al Ministerio de Educación, Cultura y Deporte.

**USO DE NANOPARTICULAS PARA LA DETERMINACIÓN DE DOPAMINA EN ORINA****J.A. Murillo Pulgarín, A. Alañón Molina, E. Jiménez García, L. García Gómez**

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La dopamina (3,4-dihidroxifeniletilamina) es una hormona endógena perteneciente al grupo de las catecolaminas. Se sintetiza en el hipotálamo y actúa como neurotransmisor, jugando un papel muy importante en el funcionamiento del sistema nervioso central, así como en el sistema cardiovascular, renal y hormonal. La detección de la concentración de dopamina resulta crucial para diagnosticar enfermedades como el Parkinson, esquizofrenia y anorexia entre otras. En fluidos biológicos el rango dinámico es muy amplio, ya que su concentración varía desde 0.1  $\mu\text{M}$  hasta 1.0 mM. Hasta ahora los métodos empleados para su cuantificación son la cromatografía líquida de alta resolución (HPLC) con la desventaja de que el equipo es costoso y necesita un protocolo especializado. El análisis electroquímico presenta una buena sensibilidad, aunque su selectividad está limitada por las interferencias de las moléculas oxidadas similares a la dopamina tales como ácido ascórbico y ácido úrico. Los métodos calorimétricos presentan una sensibilidad relativamente baja. Los métodos fluorimétricos se utilizan junto a materiales tales como puntos cuánticos, polímeros, y nanopartículas, y en la mayoría de las veces el procedimiento es complicado, lento y poco rentable. Por eso, es importante el desarrollo de un método rápido, eficiente y económico.

La dispersión de radiación resonante (RSL) es una técnica ampliamente utilizada para medir el tamaño y la distribución de partículas de polímeros, coloides, etc. Por otro lado, las señales de esta técnica se pueden mejorar cuando se aplica un haz de luz con una longitud de onda cercana a la región de absorción de las especies excitadas, donde se han encontrado numerosas aplicaciones en bioensayos y procesos de agregación, aplicaciones analíticas en el campo de la bioquímica y de la industria farmacéutica.

En el presente trabajo, se propone la determinación de dopamina mediante la inhibición que produce ésta al interaccionar con nanopartículas de conversión ascendente (UCNPs) en la medida de dispersión de radiación resonante. Para ello se sintetizaron UCNPs mediante el método hidrotérmico descrito en bibliografía<sup>[1]</sup> con algunas modificaciones.

La dopamina puede oxidarse a quinona y ésta a su vez a dopamina-melanina a través de reacciones complejas de reticulación. Cuando se ponen en contacto las UCNPs con dopamina en tampón Tris-HCl (pH=8.5), se une a la superficie de las nanopartículas mediante enlaces de hidrógeno e interacción electrostática. Los compuestos intermedios activos (quinona) y el producto final (dopamina-melanina) pueden actuar como aceptores favorables sobre la superficie de las UCNPs<sup>[2]</sup>. El transcurso de la reacción de las UCNPs con dopamina en tampón Tris-HCl (pH= 8.5) se monitorizó mediante medidas de espectroscopía UV-Vis y RLS, concluyendo que el tiempo de medida adecuado era de 90 min. A continuación se registraron espectros tridimensionales para poder seleccionar la relación  $\lambda_{\text{em}}/\lambda_{\text{ex}}$  más adecuada, seleccionando  $\lambda_{\text{em}}/\lambda_{\text{ex}} = 1/2$ , es decir excitando a 600 nm para medir a 300 nm.

El calibrado se realizó con concentraciones de dopamina entre 0 y 300.0  $\mu\text{M}$  en presencia de 0.2 mg/mL de UCNPs y por triplicado. Los parámetros analíticos del método propuesto se calcularon mediante la teoría de propagación de errores, siendo el límite de detección de 1.62  $\mu\text{M}$ . Finalmente, el método se aplicó al análisis de la dopamina en orina, siendo necesario utilizar el método de las adiciones estándar para evitar el efecto matriz, obteniendo recuperaciones próximas al 100 %.

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**POSIBILIDADES ANALÍTICAS DE UNA NARIZ ELECTRÓNICA BASADA EN  
ESPECTROMETRÍA DE MASAS PARA LA DIFERENCIACIÓN DE PACIENTES CON  
CÁNCER DE PULMÓN E INDIVIDUOS SANOS A PARTIR DEL ANÁLISIS DE MUESTRAS  
DE ORINA Y SALIVA**

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Se presenta una metodología de análisis basada en la obtención de señales de perfil con una nariz electrónica con detector de espectrometría de masas. La configuración instrumental se basa en el acoplamiento de un generador de espacio de cabeza a un espectrómetro de masas cuadrupolar a través de una columna cromatográfica que se mantiene a alta temperatura, 250 °C, durante el tiempo del análisis para eliminar su capacidad de separación.

Las señales de perfil correspondientes a las muestras de orina y saliva de los pacientes y sujetos de control se sometieron a estudios de tipo cualitativo y cuantitativo para comprobar las posibilidades de discriminación entre ambos tipos de individuos.

En el caso del análisis cualitativo [1,2], se utilizaron técnicas quimiométricas tanto de reconocimiento de pautas no supervisadas como supervisadas y, en la mayoría de los casos, se obtuvieron resultados satisfactorios tanto de sensibilidad como de especificidad. Los tratamientos quimiométricos necesarios para la diferenciación de muestras fueron más sencillos cuando se utilizaron las muestras de orina debido a que contienen un mayor número de compuestos volátiles en relación con las muestras de saliva y esto aumenta la cantidad de información disponible en la señal de perfil.

Para el análisis cuantitativo [3-5], las señales de perfil se sometieron a calibración multivariante. Se estudiaron los siguientes biomarcadores: benceno, 3-metil-1-butanol, tolueno, estireno, o-xileno, propilbenceno, 1,2,4- trimetilbenceno, 2-etil-1-hexanol, 2- butanona, 2-pentanona, pirrol, y 2-heptanona. En el caso de las muestras de saliva, la presencia de los biomarcadores se relaciona con la enfermedad ya que en los controles no se detectó ninguno. En el caso de las muestras de orina, los biomarcadores estudiados están presentes en ambos tipos de sujetos en diferente concentración. Con el objetivo de clasificar las muestras, se utilizó el test de rangos y signos de Wilcoxon comparando la concentración obtenida con la mediana de un grupo de referencia de individuos sanos. Todas las muestras se confirmaron con análisis cromatográfico y los resultados fueron similares.

En vista de los resultados obtenidos, la información contenida en las señales de perfil puede ser suficiente para la diferenciación de muestras y la metodología puede ser adecuada, en una primera etapa, como criba rápida de un gran número de muestras debido a su elevada velocidad de análisis.

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## NIR SPECTROSCOPY AS A CLEAN TOOL FOR URINE ANALYSIS

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Analysis of human blood and urine is of the many ways to evaluate people's health and to detect alterations of their health.

At present, these analysis are made using so expensive instrumentation and requires the use of specific reagents and a high cost.

In this work we have evaluated the use of direct transmittance NIR measurement of urine samples to determine different parameters of interest. 208 urine samples from different types of patients were employed, using reference values provided by the hospital laboratory. Transmittance NIR spectra were obtained using a micro flow cell with an optical path length of 1mm and a blank of water as a background. Samples were divided in a calibration and a validation set and different spectral ranges and pre-processing techniques have been considered in order to build the best calibration models by partial least squares regression and cross-validation approach.

Urea and creatinine are two of the parameters studied and, as it can be seen from the results of the table, a good predictive capability was obtained, being results for urea better than those for creatinine, probably due to the limited sensibility of the technique and the fact that creatinine concentration in urine is clearly lower than urea.

These preliminary results evidence that NIR spectroscopy can be a promising technique for a fast, direct, non-destructive and clean determination of clinical urine parameters like urea and creatinine.

		Calibration		Validation			
Analyte	LV	RMSEC (%) (m/v)	R <sup>2</sup> cal	RMSECV (%) (m/v)	R <sup>2</sup> cv	RRMSEP (%)	RPD
<i>Urea</i>	4	0.33	0.9950	0.38	0.9933	2.9	6.27
<i>Creatinine</i>	10	6.85	0.9657	12.77	0.9713	10.16	3.24

**Acknowledgements.**

Authors acknowledge the financial support of Ministerio de Economía Competitividad-Feder (Project CTQ 2014-52841-P) and Generalitat Valenciana (Project PROMETEO 11/2014/077).

## A COLORIMETRIC SENSOR FOR THE ON-SITE DETECTION AND QUANTIFICATION OF KETAMINE IN ILLICIT DRUG SAMPLES

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The production, commercialization and consumption of illicit drugs such as ketamine remain today a big problem in many countries. Although there are several analytical methodologies for drug testing, the development of rapid and cost-effective analytical tools that allow on-site detection is a challenge for forensic and toxicological laboratories. As a contribution in this area, a colorimetric sensor has been developed for the presumptive detection of ketamine in illicit drug samples. The sensor has been prepared by immobilized the reagent  $\text{Co}(\text{SCN})_2$  into polydimethylsiloxane (PDMS) [1].

When exposed to solutions of ketamine at a basic pH, the sensor color changes to blue-purple due to the diffusion of the analyte molecules to the polymeric matrix. The sensor enables the visual identification of amounts of drug as low as 30  $\mu\text{g}$  in a few minutes. Quantification of ketamine is also possible through the measurement of the absorbance in diffuse reflectance mode. Under the proposed conditions, linear responses were obtained up to concentrations of the ketamine of 1000  $\mu\text{g}/\text{mL}$  with satisfactory precision (relative standard deviations, RDSs < 10 %). The reliability of the developed sensors has been tested by analyzing illicit drug samples suspected to contain ketamine. The tested samples were also processed by Fourier transform infrared-attenuated total reflectance (FTIR-ATR) and liquid chromatography (LC) for comparison purposes.

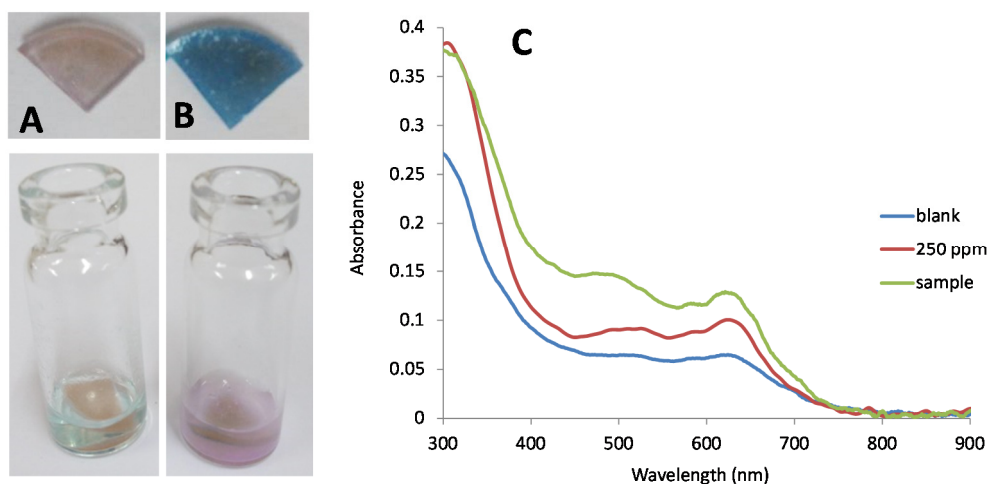


Figure 1. Sensors during (down) reaction and after reaction (up) exposure to a blank (A) and to a solution of ketamine (B); C sensor absorbance spectra registered for a blank, standard solution of ketamine (500  $\mu\text{g}/\text{mL}$ ) and sample solution.

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**DESIGN AND CHARACTERIZATION OF AN ELECTROCHEMICAL MICROBIAL SENSING  
MODULE BASED ON HYBRID BIOFILMS**

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The identification and quantification of bacterial pathogens has become a key point in biodefense, food safety, diagnostics, and drug discovery research. Bacterial infection is a common cause of morbidity and mortality worldwide, and in many cases, these infections are misdiagnosed. Thus, there is an urgent demand for rapid, cost-effective, and sensitive tests which can identify whole bacteria in the field or at the point of care.

Recent interest in lab-on-a-chip technology has been the motivation for developing miniature, accurate and rapid instrumentation to identify various strains of bacteria. This minimizes the necessity of traditional time consuming protocols in a laboratory environment. In this context, the present work shows preliminary results towards the design and characterization of heterofunctional polyelectrolytes multilayers integrating nanomaterials and immunochemistry for selective electrochemical sensing micro-devices. The proof-of-concept is being demonstrated with *E. coli* and *S. aureus*. Optical and impedimetric techniques are used for characterization whilst operating performance aims at a multisensory modular system “quasi-reagentless”, fast, and cost-effective for its further integration into a microfluidic system.

Financial support from the Spanish Ministry of Economy and competitiveness (CTQ2014 54553-C3-3-R) is acknowledged.

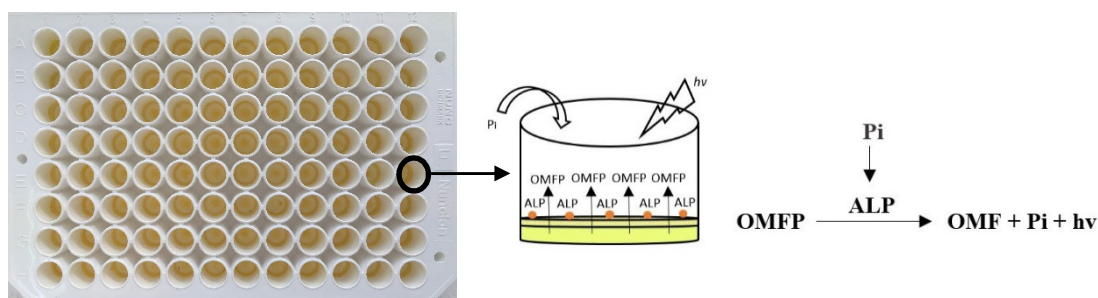
## PHOSPHATE DETERMINATION IN SERUM SAMPLES BY USING FLUORESCENT SOLID BIOSENSOR

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Inorganic phosphate concentration in serum has importance in human health because it has a critical role in numerous normal physiologic functions including energy metabolism, bone mineralization and intracellular signal. The inorganic phosphate levels in serum are regulated by intestinal absorption and renal excretion. The levels of phosphorus in serum are between 3.4-4.5mg/dL according to World Health Organization (WHO). Some pathological conditions, such as chronic kidney disease, hyperthermia, rhabdomyolysis or hyperparathyroidism among other diseases, could modify the physiological inorganic phosphate concentration in serum [1].

The demands of sustainable green methodologies have increased the use of sensors and biosensors technologies in analytical chemistry. Over the last decades, biosensors have emerged as a rapid and simple method for compounds detection in medical area, food industry and quality control. The main reason to develop sensors is to achieve the integration of multiple processes in only one device, thus the time process will be reduced [2]. In this study, a solid biosensor based on the immobilization of several reagents in a membrane of zein has been developed [3]. The dispositive is based on the fluorescent detection of 3-O-methylfluorescein (OMF) produced by dephosphorylation of OMFP carried out by the enzyme alkaline phosphatase ALP. The ALP is inhibited in the presence of phosphate ion. Several layer configurations and immobilization strategies have been tested in order to develop a reliable biosensor. The analytical proprieties of procedure have been established, being the linear range between 0.5mg/ml and 5mg/ml and the detection limit was 0.1mg/ml. This developed biosensor has demonstrated to be reliable for phosphate determination in real serum samples, is environmentally friendly and low cost device which allow the easy portability and reduce the measure time.



The authors are grateful to Generalitat Valenciana (PROMETEO 2016/109) and to the Spanish Ministerio de Economía y Competitividad (MINECO) and EU-FEDER (project CTQ2014-53916-P) for financial support. S.B.R. expresses her gratitude to MINECO/FEDER for her doctoral grant.

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**APLICACIÓN DE LA MICROEXTRACCIÓN CON ADSORBENTES ENPAQUETADOS (MEPS) AL ANÁLISIS DE SUSTANCIAS PSICOTRÓPICAS EN MATRICES BIOLÓGICAS**

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La preparación de la muestra es una etapa fundamental en la Química Analítica, sobre todo cuando se trabaja con matrices biológicas complejas, como la sangre, orina o fluido oral, muy usadas en toxicología forense. El objetivo de esta preparación es el aislamiento y concentración de los analitos de interés a partir de la matriz compleja. Con ello se consigue una limpieza eficaz (etapas de *clean up*), reducción del efecto matriz y mejora de la sensibilidad de la etapa analítica instrumental[1]. Una de las técnicas más utilizadas es la extracción en fase sólida (SPE)[2], pero en las últimas décadas se ha introducido la microextracción con adsorbentes empaquetados (MEPS), una miniaturización de la SPE en la que se reducen la cantidad de muestra, el volumen de disolventes, el tiempo de extracción y el coste, además de la reutilización de la fase adsorbente [3].

El informe europeo sobre drogas del año 2016[4] pone de manifiesto la aparición de nuevas sustancias psicotrópicas en el mercado y el aumento del consumo de las sustancias ya existentes, cada año. Por este motivo, es necesaria la puesta a punto de nuevas técnicas analíticas que permitan la identificación y la cuantificación rápida y precisa de todas estas sustancias en distintas matrices. La microextracción con adsorbentes empaquetados permite el análisis de estas sustancias de forma más rápida y económica que otras técnicas de extracción convencionales.

Esta comunicación tiene como objetivo la presentación de diversos protocolos de MEPS para la determinación de distintas sustancias psicotrópicas en tres tipos de matrices biológicas de interés toxicológico. Se optimizaron y estudiaron distintos parámetros que afectan al proceso de extracción: tipo de fase adsorbente utilizada, volumen de muestra, número de emboladas empleadas durante el proceso de carga y descarga de la muestra, pH de la muestra, tipo y volumen de disolvente, tanto de elución como de lavado de la fase. Ante el número de variables a estudiar, se empleó una técnica quimiométrica como es el diseño de experimentos, permitiendo la reducción del número de experiencias a realizar y de los recursos a emplear (tiempo y material).

**Agradecimientos**

Los autores agradecen a la Dirección General de Tráfico Ministerio del Interior, (España), Proyecto SPIP2015-01838, y al Ministerio de Economía Industria y Competitividad (Proyecto AGL-2014-53647-R) y fondos FEDER la financiación de este trabajo.

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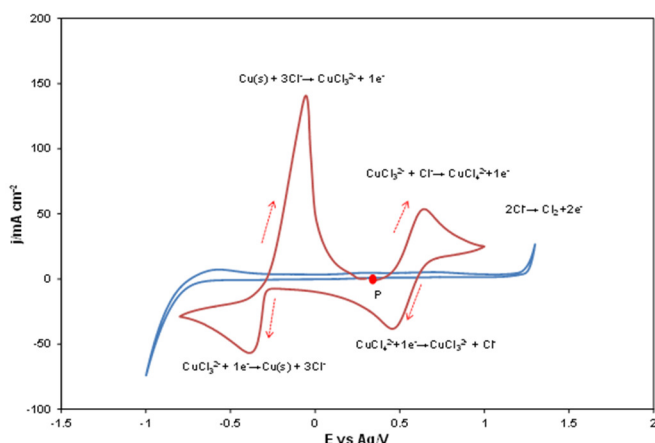
# COMPORTAMIENTO ELECTROQUÍMICO DEL Cu EN EL DES ("DEEP EUTECTIC SOLVENT") CLORURO DE COLINA- ETILENGLICOL 1:2 SOBRE ELECTRODO DE CARBONO VÍTREO

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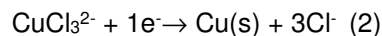
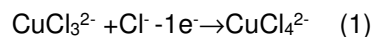
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Las mezclas eutécticas, que son líquidas a temperatura ambiente y poseen características físico-químicas similares a los líquidos iónicos (ILs) son más económicas y menos agresivas con el medio ambiente que éstos, por lo que se constituyen como medios alternativos para estudios electroquímicos [1]. Las más comunes consisten de la mezcla de cloruro de colina (ChCl) con una especie donadora de enlaces de hidrógeno (HBD), como aminas, ácidos carboxílicos, glicoles y fenoles. Entre las especies HBD más utilizadas para la formación de depósitos metálicos y aleaciones se encuentra el etilenglicol (EG).



En esta comunicación se presenta el estudio del comportamiento electroquímico de los iones Cu(I) sobre electrodo de Carbono vítreo (GC) en el DES ChCl-EG 1:2 a 333,15 K.

La electro-oxidación y electro-reducción de CuCl<sub>3</sub><sup>2-</sup> sobre GC tiene lugar de acuerdo a las siguientes reacciones electroquímicas:



Dichas reacciones tienen lugar a valores de potencial suficientemente

separados que garantizan la estabilidad de la especie CuCl<sub>3</sub><sup>2-</sup>.

El coeficiente de difusión de la especie CuCl<sub>3</sub><sup>2-</sup> se ha determinado por diversas técnicas electroanalíticas aplicadas tanto al sistema Cu(I)/Cu(II) como al Cu(I)/Cu(s), observando que no existen diferencias significativas entre los valores obtenidos.

**Sistema Cu(I)/Cu(II):** Se han determinado, por primera vez en este medio, los valores de la constante de velocidad intrínseca de transferencia de carga  $k^0$  y del coeficiente de transferencia de carga  $\alpha$ , mediante simulación de los voltamperogramas cíclicos y análisis logarítmico de las curvas convolucionadas y voltamperogramas.

**Sistema Cu(I)/Cu(s):** Se ha caracterizado la nucleación y crecimiento cristalino de cobre sobre carbono vitrificado mediante cronoamperometría. Las curvas adimensionales I-t se ajustaron a un modelo de nucleación instantánea.

**Agradecimientos:** Los autores agradecen a la Junta de Castilla y León (proyecto VA171U14) la financiación prestada.

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## COMPORTAMIENTO ELECTROQUÍMICO DEL Ni EN EL NADES ("NATURAL DEEP EUTECTIC SOLVENT") CLORURO DE COLINA- ETILENGLICOL 1:2

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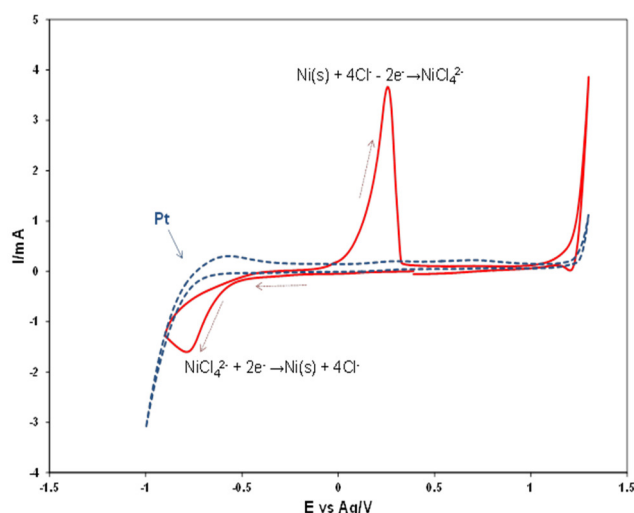
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Las mezclas eutécticas (DES) son medios cuyo punto de fusión es muy inferior al de sus componentes por separado. En general son líquidos a temperatura ambiente y poseen características físico-químicas similares a los líquidos iónicos (ILs), aunque son más económicas y menos agresivas con el medio ambiente que éstos, por lo que se constituyen como medios alternativos para estudios electroquímicos [1]. Los DES más utilizados consisten en la mezcla de cloruro de colina (ChCl) - aceptor de enlaces de hidrógeno (HBA)-, con una especie donadora de enlaces de hidrógeno (HBD). Cuando los compuestos que constituyen el DES son metabolitos primarios (aminas, ácidos carboxílicos, etc.) se las denomina NADES (Natural Deep Eutectic Solvents) [2]. Entre las especies HBD más utilizadas para la formación de depósitos metálicos y aleaciones se encuentra el etilenglicol (EG) [3].

Los electrodepositos de níquel se utilizan ampliamente por su resistencia a la corrosión, aplicaciones decorativas y en la fabricación de circuitos electrónicos impresos. Por estas razones en esta comunicación se presenta el estudio del comportamiento electroquímico de  $\text{NiCl}_2$  disuelto en el "NADES" ChCl-EG 1:2 "etalina" a 343 K sobre Pt, así como la obtención de depósitos de níquel mediante electrólisis (sobre Pt) y reacciones de sustitución galvánica con Fe y Cu como metal de sacrificio.

Las disoluciones de  $\text{NiCl}_2$  en Etalina presentan termocromismo presentando un color verde-amarillento a 308, verde a 343 y azul a 393K.



La reducción de Ni(II) a Ni(0) sobre Pt tiene lugar mediante un proceso irreversible.

Se ha determinado el coeficiente de difusión de Ni(II) por diversas técnicas electroanalíticas, y se ha observado que la nucleación y el crecimiento cristalino de Ni juega un papel fundamental en el proceso de electrodeposición.

Finalmente se ha estudiado el proceso de obtención de depósitos electroquímicos de Ni, así como reacciones de sustitución galvánica de cobre con níquel y de hierro con níquel.

**Agradecimientos:** La autores agradecen a la Junta de Castilla y León (proyecto VA171U14) la financiación prestada.

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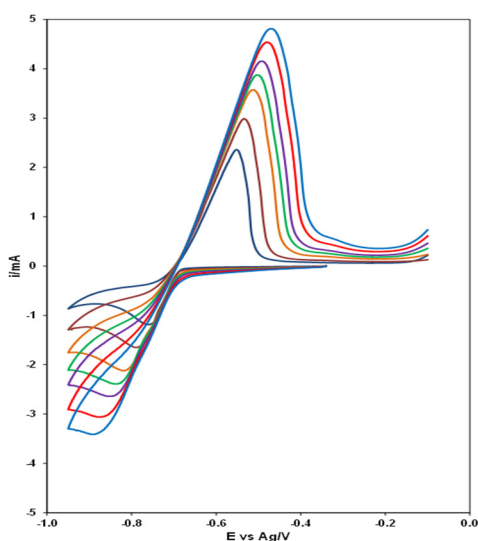
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## COMPORTAMIENTO ELECTROQUÍMICO DEL Cd EN CLORURO DE COLINA-ETILENGLICOL Y DE LA CORROSIÓN DE SUS DEPÓSITOS SOBRE DIVERSOS SOPORTES

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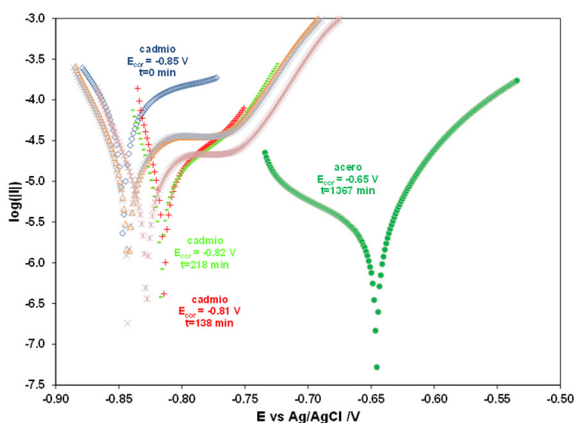
En esta comunicación se lleva a cabo un estudio del comportamiento electroquímico del Cd (II) en el NADES [1,2] Cloruro de colina-etilenglicol (ChCl-EG), 1:2 sobre diversos soportes electroquímicos (platino, hierro, acero al carbón 1018, etc) a temperaturas comprendidas entre 333 y 363 K.

Se ha determinado el coeficiente de difusión (D) mediante voltamperometría cíclica (CV), cronoamperometría (CP) y cronoamperometría (CA).

Para caracterizar los fenómenos de nucleación y crecimiento cristalino de cadmio se realizó un análisis cronoamperométrico sobre electrodos de trabajo de Pt, hierro y acero al carbón. En todos los casos las curvas adimensionales I-t se ajustaron a un modelo de nucleación

instantánea

Posteriormente se procedió a la formación de depósitos metálicos sobre los distintos soportes con el fin de analizar el fenómeno de corrosión en un medio salino (NaCl 3% peso). La evolución de los depósitos se realizó mediante la intersección de las porciones rectas, anódica y catódica de las pendientes de Tafel obtenidas por polarización potenciodinámica.



**Agradecimientos:** La autores agradecen a la Junta de Castilla y León (proyecto VA171U14) la financiación prestada.

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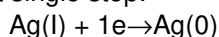
# ELECTROCHEMICAL BEHAVIOUR OF Ag (I) AT Pt ELECTRODE IN THE DEEP EUTECTIC SOLVENT CHOLINE CHLORIDE: 2 ETHYLENGLYCOL AT 343-363 K

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The electrochemical reduction of Ag (I) on a platinum electrode has been studied in the Deep Eutectic Solvent (DES) Choline Chloride: 2 Ethylenglycol (ChCl:2EG) at 343-363 K, by square wave voltammetry (SWV), cyclic voltammetry (CV), convolutive potential sweep voltammetry (CPSV), chronoamperometry (CA), and chronopotentiometry (CP).

It has been found that during cathodic polarization, deposition of metallic Ag from the ChCl-EG onto the platinum surface proceeds in a single step:



which has been found reversible or quasi-reversible depending on the experimental conditions (i.e scan rate).

The diffusion coefficient of Ag(I) (D) has been determined by different techniques and compared with those reported in the literature in other Ionic Liquids (1). The validity of the Arrhenius law was also verified.

It has been found that electro-crystallization of silver on the Pt substrate plays a role at the very first potential of the electrodeposition process.

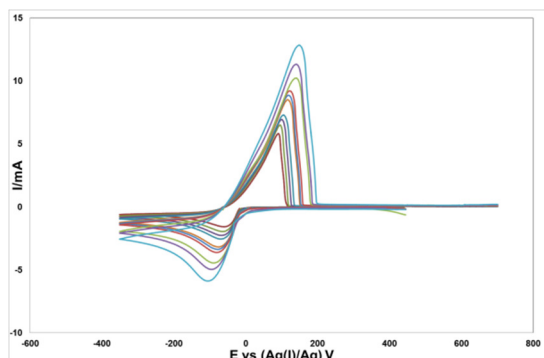


Figure 1: Cyclic Voltammograms obtained with an Ag(I) solution ( $C_0 = 7.43 \cdot 10^{-5} \text{ mol cm}^{-3}$ ,  $T = 363 \text{ K}$ ) on a Pt electrode ( $S = 0.42 \text{ cm}^2$ ). Scan rates ranging from 20 to  $300 \text{ mV s}^{-1}$ .

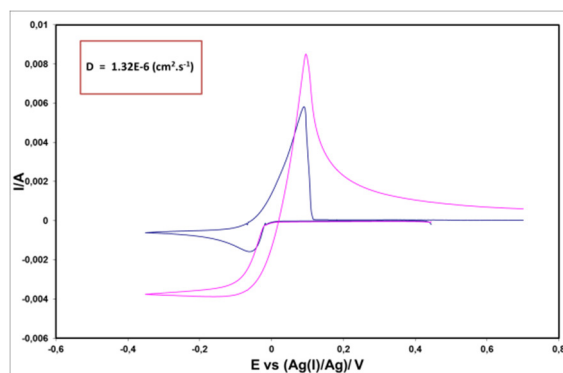


Figure 2.- Cyclic Voltammogram and convoluted curve.

Acknowledgements: Authors thank the Junta de Castilla y León Project VA171U14 for the financial support

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# **ELECTROCHEMICAL IDENTIFICATION OF ARQUEOLOGICAL STRATA: VIMP ANALISYS OF BRONZE COINS FORM THE MAGNA MATER TEMPLE**

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The Magna Mater Temple, dedicated to the Phrygian divinity Cybele, was inaugurated in 191 B.C. Lately it was destroyed by fire twice and rebuilt by Augusto in 3 A.D. The site, after the temporary abandonment in 410 A.D., was reused until the 17th century A.D. These historical data conjointly with archaeological findings, together with the stratigraphy, have contributed to the elaboration of a coherent periodization aimed at establishing timelines, original usage, changes and final destination of the site in which the coins were found. In particular, the coins of the Magna Mater Temple cover the entire Imperial Roman period, becoming of great relevance for the history of the site and for the historical questions related to the reuse of the materials. Regarding the stratigraphy of the site, the coins resulted older than the deposition's date, gathering around the first half of the 4<sup>th</sup> century. The archaeologists explained this chronology as resulting from the prolonged use of the Roman emissions. The aim of this study was to evaluate the possible existence of different types of coins, especially for age and/or provenance and/or stratigraphy, on the basis of the analysis of the electrochemical response of the patina. In order to achieve these, the coins were studied using the voltammetry of immobilized particles (VIMP) methodology, a technique which provide responses depending on the composition of the base metal (alloy of copper, tin and lead). Characteristic voltammetric patterns of corrosion products were recorded: cuprite in the primary and secondary corrosion patina and tenorite in the secondary patina for submicrosamples of the corrosion layers of coins. The ratios between different pairs of peak currents recorded under fixed electrochemical conditions were used for grouping the coin samples into three main groups, corresponding to different archaeological strata [1]. The electrochemical data can be considered as consistent with the hypothesis of the reuse of the coins during the later periods as a result of the economic difficulties associated to the fall of the Roman Empire. In conclusion, the discrimination of the coins, which depends on their usage history, allows the grouping of the studied samples on the basis of their location in the stratigraphic sequence of the archaeological site. Taking into account the highly non-destructive and non-invasive characteristics of the proposed electrochemical methodologies, this approach can be a useful tool for archaeologists in the future.

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# IDENTIFICATION OF SULFUR-CONTAINING COMPOUNDS IN ASPHALT CEMENTS BY ELECTROCHEMICAL METHODS IN SOLID STATE

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Determination of organosulfur compounds in asphalt cements is important from scientific and technological view-point, since sulfur species are considered precursors of the asphalt oxidation reaction [1]. The solution-phase electrochemical methods have been applied to petrochemical analysis and were originally used in the analysis of sulphur and sulphur-containing compounds. Moreover, it has been used to monitor the efficiency of a cleanup method for the determination of organosulfur compounds in asphalt cements [2]. Voltammetry of immobilized microparticles is a solid-state electrochemical technique and through potential scanning and subsequent recording of a current signal, is able to provide valuable information about the kinetics of oxidation reactions of organosulfur compounds on asphalts [3,4]. In this way, cyclic and square wave voltammetries were used as detection modes employing 0.10 M H<sub>2</sub>SO<sub>4</sub> as a supporting electrolyte to determine sulphides, thiophenes, sulfoxides and sulfones. The working electrode consisted of paraffin-impregnated graphite modified with the compounds and asphalt samples the reference and auxiliary electrodes consisted in Ag/AgCl (3M NaCl) and Pt, respectively. Under the used experimental conditions, benzothiophenes (4-methyl dibenzothiophene, 4,6- dimethyl dibenzothiophene, 2-phenyl thiophene, 3-phenyl thiophene, thianthrene, thianaphthene, dibenzothiophene, dibenzyl sulphide) produced two oxidation peaks around +1.35 V and +1.75 V. In the same way, sulfoxides (methyl phenyl sulfoxide and dimethyl sulfoxide) were oxidized and demonstrate two oxidation peaks around +1.15 V and +1.75 V. Sulfones (allyl phenyl sulfone, benzyl sulfone, dimethyl sulfone, di-p-toluyl sulfone) were previously electrolysed by 300 s under -1.25 V and the anodic scan demonstrated the presence of different reduction products generated during the electrolysis. Moreover, the sulfones displayed cathodic signals overlapped with the proton discharge around -1.35 V. Such processes can be described in terms of proton-assisted solid-state electron transfer processes in agreement with theoretical approaches for VMP measurements [3,4]. The asphalt cements were provided by PETROBRAS (CENPES, BRASIL) in different conditions (aged and un-aged) and it was possible to identify the presence of organosulfur compounds in the samples by VIMP. Furthermore, the samples demonstrated better signal intensities for sulphur-containing compounds in the reduced form (thiophenes and sulfides) in the un-aged samples than the aged ones. In the same way, sulphur-containing compounds in the oxidized form demonstrate more intensity of the signals in the aged samples, which could demonstrate the involvement of such species in the aging process through the oxidation of thiophenes and sulfides to sulfoxides and sulfones.

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# **EVALUATION OF TRANSDUCTION STRATEGIES USING ENZYME OR QUANTUM DOTS AS LABELS IN THE DEVELOPMENT OF MAGNETOGENOSENSORS FOR THE DETERMINATION OF A TRANSCRIPTION FACTOR RELATED TO CANCER**

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Nowadays, there is a great interest in the development of simple and reliable electrochemical systems for routine determinations of clinical biomarkers and successful implementation of point-of-care (POC) devices. A number of these systems are based on the use of superparamagnetic iron oxide particles (MBs) functionalized with different reactive groups, that allow selective, rapid and easy capturing of specific target molecules from complex samples, and are easily coupled with disposable electrochemical sensors. Several transduction systems have been used in the amplification and quantitation protocols coupled with these magnetic sensors, the main objective of this work relying on the comparison of two of them.

The genes of the sex determining region Y-BOX (SOX) encrypt a family of high mobility group (HMG) from the transcription factor (TF) family. Around 20 genes of the SOX family have been defined and classified depending on their protein specificity. Among them, SOX-2, which is closely related to the early embryonic development, neural and sexual differentiation, has been demonstrated to be amplified and overexpressed in various malignant tumors.

Herein, the coupling of biotinylated DNA hybrids, formed by hybridization between a synthetic specific capture probe immobilized onto MBs and a complementary sequence including a specific fragment of the target SOX-2 gene, either with a commercial streptavidin-peroxidase (Strep-HRP) conjugate to perform amperometric detection of the hybridization step using the hydrogen peroxide/hydroquinone ( $\text{H}_2\text{O}_2/\text{HQ}$ ) system, or with streptavidin-modified CdSe/ZnS quantum dots (QDs) followed by their square wave anodic stripping voltammetric (SWASV) detection, is evaluated in terms of sensitivity, reproducibility, reliability and other characteristics of the developed DNA sensors. Finally, the development of a magnetogenosensor for the determination of the SOX-2 cancer marker coding sequence involving the use of Strep-MBs, a specific biotinylated capture probe and enzymatic amperometric detection at disposable screen-printed carbon electrodes (SPCEs) will be described. Under optimal conditions, this amperometric magnetogenosensor demonstrated a wide linear concentration range (2.5 pM –1 nM) and a detection limit as low as 0.8 pM for the synthetic SOX-2 specific DNA target. Furthermore, the developed SOX-2 magnetogenosensor had good storage stability, the capture probe modified MBs being stable for at least 10 days, and high reproducibility (RSD 2.8 %,  $n = 10$ ) between the amperometric responses provided by different magnetogenosensors prepared in the same manner. Moreover, the implemented DNA platforms gave amperometric responses only slightly higher than the signal measured in the absence of target DNA for fully non-complementary and 2-mismatch oligonucleotide sequences, thus confirming the good selectivity of the developed magnetogenosensor. These characteristics allowed the determination of the target SOX-2 sequence directly in complex non-invasive biological samples such as saliva in less than 2 h, thus offering an alternative diagnosis tool for the early diagnosis and prediction of certain types of cancer.

## ESTUDIOS VOLTAMPEROMÉTRICOS DE HISTAMINA Y TIRAMINA EMPLEANDO UN ELECTRODO DE CARBÓN VITRIFICADO

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Las aminas biógenas son compuestos básicos nitrogenados con actividad biológica que se encuentran en los alimentos y bebidas, formados principalmente por la descarboxilación de aminoácidos. Debido a su relevancia para la salud humana, la concentración de aminas biógenas ha sido propuesto como parámetro de calidad, de ahí la importancia de su determinación.

Algunos de los métodos desarrollados para la determinación de dichos analitos involucran técnicas separativas. Sin embargo, ya que no presentan grupos cromóforos es necesario someterlos a una reacción de derivatización para poder detectarlos mediante espectrofotometría de absorción molecular UV-Vis así como fluorescencia molecular. Para evitar todos los problemas relacionados con las reacciones de derivatización, se puede recurrir a las técnicas electroanalíticas para su cuantificación, ya sea por voltamperometría o por técnicas separativas con detección electroquímica, ya que estos compuestos son susceptibles de ser oxidados mediante la aplicación de un potencial eléctrico sin necesidad de recurrir a los procesos de derivatización [1-2].

Se ha llevado a cabo un estudio electroanalítico básico de dos de estos compuestos, concretamente, histamina y tiramina, mediante técnicas voltamperométricas utilizando un electrodo sólido como electrodo de trabajo, el electrodo de carbono vitrificado, para ver la posibilidad de poderlos determinar posteriormente de forma conjunta empleando un electrodo de trabajo económico.

Para la limpieza del electrodo se probaron cuatro procedimientos, siendo la limpieza mecánica seguida de una limpieza electroquímica, el método óptimo para obtener medidas reproducibles de ambos analitos. En el estudio de pH, empleando disolución Britton-Robinson, se emplearon las técnicas voltamperométricas diferencial de pulso, voltamperometría de corriente continua, voltamperometría de onda cuadrada y voltamperometría cíclica. Los valores óptimos encontrados para la histamina y la tiramina fueron pH 6.0 y 3.0, respectivamente.

Finalmente, se estudió la influencia de la concentración, observándose el fenómeno de saturación del electrodo. Se estableció el intervalo lineal, así como los parámetros de calidad para ambos analitos (sensibilidad analítica, límites de detección, etc.). Se realizó también un estudio de la repetitividad entre las medidas efectuadas, obteniéndose un error del 4,06% y 4,02% para histamina y tiramina, respectivamente.

**Agradecimientos.** Los autores agradecen la financiación de este trabajo al Ministerio de Economía y Competitividad de España (Proyecto CTQ2014-52309-P) y a la Junta de Extremadura (Proyectos IB16058 y GR15090-Grupo de investigación FQM003). Todos los proyectos están cofinanciados por los Fondos Europeos FEDER.

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## ESTUDIO DE LA EFICIENCIA DEL ELECTRODO APLICADO A ELECTROMEMBRANA COMO PROCEDIMIENTO DE PRE-TRATAMIENTO DE MUESTRA

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El empleo de electromembranas como procedimiento para el pre-tratamiento de muestras ha cobrado especial interés en los últimos años. La extracción mediante electromembranas en tres fases favorece la migración de los analitos desde una fase donadora/muestra (acuosa) hasta una fase aceptora (acuosa) a través de la membrana líquida soportada, gracias a un campo eléctrico generado cuando se aplica una diferencia de potencial entre ambas fases.

La extracción mediante electromembranas se aplica empleando sistemas de diferente geometrías. Los factores que afectan a la electromembrana son la naturaleza del disolvente orgánico, la composición y el pH de la fase donadora y aceptora, y el voltaje aplicado. Dependiendo del tipo de geometría y de la modalidad de trabajo, podrán depender de otros factores como son la velocidad de agitación, el flujo tanto de la fase donadora y aceptora, etc.

Hasta el momento no se ha tenido en cuenta la importancia de las características del electrodo, ni se ha realizado un seguimiento de la estabilidad y eficacia del mismo previo a su aplicación en electromembrana. Por ello se pone a punto un procedimiento de caracterización del electrodo para estudiar cómo afecta en procedimientos de electromembrana. El estudio se realiza empleando dispositivos microfluídicos<sup>1</sup> y utilizando un electrodo cilíndrico de platino de 100 µm de diámetro externo y 20 mm de longitud. La caracterización del electrodo se realiza mediante voltamperometría cíclica y un estudio de impedancia, empleando para ambos la pareja redox ferri/ferrocianuro equimolar, y técnicas de microscopía SEM sobre el electrodo.

En este trabajo se presentan las diferentes recuperaciones y eficiencias de extracción obtenidas cuando se aplica la electromembrana a una serie de analitos empleando electrodos nuevos, reutilizados y reactivados. La reactivación del electrodo se consigue empleando una disolución de 0,1M de  $KNO_3$ .

**Agradecimientos:** BP B-Marie Curie (0025) (the 7th Framework Programme European Commission) y el Ministerio de Economía, industria y competitividad- contrato Juan de la Cierva Incorporación.

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**DETERMINACIÓN DE ARSENICO CON ELECTRODOS DE PASTA DE CARBON MODIFICADO CON *LESSONIA NIGRESCENS* (HUIRO NEGRO) O ÁCIDO ALGÍNICO EXTRAÍDO DE ALGAS PARDAS.**

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*Lessonia nigrescens* (Huiro negro) es una alga muy abundante en las costas de Chile. Se utiliza como fuente industrial para la producción de alginatos y geles de polisacáridos utilizados en la industria de alimentos, cosméticos y pinturas. Estudios han revelado que esta alga, tiene una alta capacidad de adsorción por el arsénico de los medios acuáticos circundantes<sup>1,2</sup>. Esta inusualmente alta afinidad por los iones arsénicos, así como la facilidad y el costo de la preparación de los electrodos, hacen que las algas sean muy atractivas para la modificación de éstos y su posterior uso en la determinación de metales y no metales traza en diferentes matrices.

Luego de recolectar las algas en diferentes lugares, éstas fueron secadas, trituradas y tamizadas. En la preparación de los electrodos, se mezcló distintas proporciones (m/m) de grafito y biomasa en un mortero de ágata con aceite mineral o con parafina en su punto de fusión (sobre 56-58 °C). Se agregaron 2 ó 3 gotas del aceite o su equivalente de parafina y se mezcló con una espátula hasta obtener una pasta homogénea. Posteriormente esta pasta se vertió en un tubo cilíndrico de 4 mm de diámetro y se introdujo un alambre de cobre para hacer el contacto eléctrico. De igual forma se preparó electrodos de pasta de carbón con distintas proporciones de grafito y alginato. Este reactivo tiene diferente viscosidad ( $\approx 250$  cps y 20-40 cps) y se obtuvo mejores resultados con el obtenido de algas marrones ( $\approx 250$  cps). También se preparó electrodos de carbono vítreo y serigrafiados de carbono incorporando el alginato mezclado con nafion. Otra metodología consistió en introducir estos electrodos (con o sin nafion) en una disolución que contenía 0,4 mg/mL de ácido algínico y se realizó electrólisis durante 15 min. a -0,70 V (1500 rpm). Se comparó resultados al incorporar películas de metales en los electrodos. Se aplicaron las técnicas de Redisolución anódica (ASV) y de Voltamperometría de Adsorción. Con el objetivo de obtener una metodología sensible, se realizó un estudio en función del electrólito soporte, pH, potencial y tiempo de acumulación ( $E_{acc}$ ,  $t_{acc}$ ) y parámetros del barrido.

Con el electrodo de pasta de carbón modificado con huiro negro se obtuvo mayores corrientes al mezclar grafito con huiro negro (zona de Chañaral) en proporción 60:40 y las condiciones elegidas fueron pH 2,0 (tampón Britton Robinson),  $E_{acc}$  -0,60 V,  $t_{acc}$  160 s y Frecuencia 50 Hz. El rango lineal fue de 2,7 – 62,5  $\mu\text{g/L}$  y el límite de detección (LD) 1,7  $\mu\text{g/L}$ . En estas condiciones se obtiene una señal ancha a -0,45 V, sin embargo al realizar el estudio de interferencias se pudo apreciar que Pb(II) interfiere incrementando dicha señal. Se están realizando medidas en presencia de agentes enmascarantes para Pb(II).

Con el electrodo de pasta de carbón modificado con ácido algínico, se obtuvo buenos resultados al mezclar grafito y alginato en una proporción de 70:30, las condiciones óptimas fueron: pH 2,0 (disolución de  $\text{HNO}_3$ ),  $E_{acc}$  -0,90 V,  $t_{acc}$  60s; frecuencia 15 Hz. Al realizar el barrido en sentido anódico, se obtuvo una señal baja alrededor a 0,0 V (alginato) y otra muy fina y de mayor intensidad a 0,26 V, con un rango lineal entre 3,7 -30,0  $\mu\text{g/L}$  y un LD de 1,9  $\mu\text{g/L}$ . Se validó la metodología utilizando agua de mar sintética y posteriormente se analizó muestras de agua de mar de la bahía de Quintero, sin embargo, el contenido de As(III) estuvo bajo el límite de detección. Con este tipo de electrodos Pb(II) no interfiere.

Agradecimientos: Los autores agradecen el financiamiento obtenido a través del Proyecto Fondecyt Regular N° 1171189.

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## VALIDATION OF A MICROEMULSION ELECTROKINETIC METHOD FOR THE LIPOPHILICITY DETERMINATION OF ACIDIC COMPOUNDS

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Lipophilicity, which represents the affinity of a molecule for a lipophilic environment, is a relevant physicochemical property to be determined in the drug discovery and design process [1], since it plays a fundamental role on drug absorption, distribution, metabolism, excretion, and toxicity (ADMET). The lipophilicity of a compound is commonly measured by its distribution behavior in a *n*-octanol/water biphasic system. It is normally referred to unionized species, even in the case of compounds with acid/base properties, and reported as the logarithm of the partition ratio ( $\log P_{o/w}$ ). Besides its importance in drug development, given that lipophilicity is a critical parameter for chemical safety assessment, according to the REACH Regulation ((EC) No 1907/2006)  $\log P_{o/w}$  values must be reported for any organic compound produced in quantities of one tonne or more per year. Two test procedures are described in the Test Methods Regulation ((EC) No 440/2008): a direct measurement via the shake-flask method [2] and a correlation approach using the HPLC method [3]. However, other experimental methods can be used provided that they show an acceptable level of quality assurance [4].

Several chromatographic methods were proposed for lipophilicity determination, mainly using reversed-phase columns and buffered mobile phases containing acetonitrile as organic modifier [5]. Although these approaches are significantly time-saving, they normally require the introduction of molecular descriptors for hydrogen-bond acidity [5–8] and are less accurate ( $\pm 0.5$  [3]) than shake-flask methods ( $\pm 0.3$  [2]). Microemulsion electrokinetic chromatography (MEEKC) is a very interesting alternative for lipophilicity measurement, given that the oil droplets acting as pseudostationary phases are better surrogates of *n*-octanol/water systems than reversed-phase columns. In fact, MEEKC measurements can be accurately correlated with  $\log P_{o/w}$  without the need of molecular descriptors [9,10].

In the present work a high-throughput methodology for  $\log P_{o/w}$  determination of acidic compounds ( $pK_a > 3$ ) was proposed and validated using conventional CE instruments with UV detection and uncoated fused silica capillaries. The ME consisted of 1.3% (w/v) SDS, 8.15% (v/v) 1-butanol, 1.15% (v/v) heptane, and 20 mM phosphoric acid, pH 2.0. 3-methylbenzoic acid, phenobarbital, barbital, and thiouracil were proposed as calibration standards, allowing the measurement of  $\log P_{o/w}$  values in the range comprised between -1.54 and 4.01 with a prediction accuracy not worse than 0.4.

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**APPLICATION OF QbD APPROACH AND NIR TECHNOLOGY FOR THE QUALIFICATION OF A FREEZE-DRYING PROCESS****G. Clua<sup>1,2</sup>, L. Garcia<sup>1,2</sup>, S. Nikolic<sup>1</sup>, E. Jo<sup>1</sup>, J. Coello <sup>2</sup> and Maspoch S<sup>2</sup>**<sup>1</sup>*Center of Excellence in Lyophilization, Reig Jofre Group*<sup>2</sup>*Department of Chemistry, Universitat Autònoma de Barcelona, 08193 Bellaterra, [santiago.maspoch@uab.es](mailto:santiago.maspoch@uab.es)*

In the pharmaceutical industry, freeze-drying is used to transform solutions of active ingredients, with or without excipients, in sterile solid products which are more stable. The definition of a freeze-drying process that maximizes the speed of sublimation while preserving the quality of the product is of great industrial interest. In the framework of Quality by Design (hereinafter QbD) and using a risk based approach, historical data analysis was combined with new experimental studies in order to define a broader knowledge space and propose a design space for the sublimation phase of the freeze-drying process. The design space was defined as a combination and multidimensional interaction of process parameters that describes a region in which the quality of the product is preserved.

A Doehlert design of experiments was executed with 2 factors (temperature and pressure) and the sublimation time, appearance and reconstitution time were evaluated as responses. The product complies with the specifications throughout the experimental regions. Then, the results of sublimation time were adjusted to a MLR model. The model fits perfectly to a linear regression ( $R^2 = 0,996$ ) and has a high predictive capacity ( $Q^2 = 0,967$ ). Regarding the coefficients, the temperature factor is the predominant to predict the duration of the sublimation. Finally, the obtained model was used to predict the optimum conditions and justify the operational settings. The newly developed process was scaled-up to the industrial plant and a manufacturing batch at the border of the proposed design space was put into stability studies.

After the development and successful scale-up, the process was qualified. The increased number of samples for residual water analysis was handled by means of near infrared spectroscopy due to its capability to determine large number of samples in a non-invasive and non-destructive analysis, giving an accurate result and requiring minimal or no sample preparation. It was used to perform a moisture mapping to statistically assess the variability among distinct positions, shelves, and freeze-dryers, and establish the correct sampling plan during routine production. For this purpose, a near infrared model was developed and validated according to the methodology recommended in EMA guidance and ICH Q2. After, the predicted residual water content results were plotted in a 3D representation of the freeze-dryers chamber to visualize the spatial distribution of the residual vials in the vials inside the freeze-dryers. The project was developed in collaboration with the Spanish Medicines Agency (AEMPS).

**DETERMINATION OF POLYCYCLIC AROMATIC HYDROCARBONS IN  
COSMETIC PRODUCTS AND RAW MATERIALS BY  
GAS CHROMATOGRAPHY-MASS SPECTROMETRY**

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Polycyclic aromatic hydrocarbons (PAHs) are a broad family of organic compounds formed by the fusion of two or more aromatic rings. They are produced by the incomplete combustion of organic matter and fossil fuels, such as coal, petroleum and natural gas [1]. It is well-known that PAHs are hazardous for human health due to their endocrine disrupting properties and carcinogenic effects.

In addition to its use as fossil fuel, petroleum is also used as source of raw materials for the manufacture of cosmetic products, so in case that PAHs are produced during petroleum processing, they could be present in cosmetics. In consequence, the European Regulation on Cosmetic Products [2] forbids the presence of 11 PAHs. For that reason, it is necessary to have suitable analytical methods to perform adequate quality controls of both raw materials and cosmetic products, in order to assure the safety of users. Nevertheless, there are not official methods for the identification or the determination of these compounds in cosmetic products and the analytical literature regarding to the determination of PAHs in cosmetics is also insufficient.

Thus, the aim of this work is to develop and validate a simple and reliable analytical method for the determination of 11 forbidden PAHs (naphthalene, acenaphthene, anthracene, benz[a]anthracene, chrysene, benzo[j]fluoranthene, benzo[k]fluoranthene, benzo[b]fluoranthene, benzo[e]pyrene, benzo[a]pyrene, dibenz[a,h]anthracene) and other 7 hazardous PAHs (acenaphthylene, fluorene, fluoranthene, phenanthrene, pyrene, indeno[1,2,3-cd]pyrene, benzo[g,h,i]perylene) in both cosmetic products and raw materials. The method is based on gas chromatography coupled to mass spectrometry as separation, identification and quantification technique.

**Acknowledgements**

Authors acknowledge the financial suport of the Spanish Ministry of Economy and Competitiveness (Project CTQ2015-70301R). P.M. also thanks the Spanish Ministry of Education, Culture and Sports for his predoctoral grant.

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## USE OF MICROEXTRACTION TECHNIQUES FOR GREEN COSMETIC ANALYSIS

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The daily or very frequent use of cosmetic products involves the continuous exposure of their users to the ingredients. The European Regulation of Cosmetic Products (effective since July 2013) imposes clearer and more detailed rules in relation to safety than that previously existing. In order to ensure a high level of health protection, certain elements of the regulatory framework, such as market control, are strengthened. Moreover, the need to use analytical methods adopted by the 'European Committee for Standardization' (CEN) as 'harmonized standards' is established.

In the so-called EU Cosmetic Regulation, there are listed more than 1800 substances either as prohibited or as restricted in terms of concentration. However, the process of validation of methods for consideration as 'harmonized standards' is still starting. There are very few validated methods available that could be used by the companies (or specialized laboratories) for quality control, or by the health authorities to control the market. Furthermore, there are not enough published methods on this topic, and some of them require proper validation and modernization according to current advances in sample preparation and analysis.

Fast and easy to implement microextraction techniques provide green analytical methods, i.e., environmentally friendly and safety for the operator, which is really interesting due to the high number of samples to be analyzed for quality control.

In this work, several contributions of our group based on the use of microextraction techniques (e.g., dispersive liquid-liquid and vortex assisted liquid-liquid) for the determination of cosmetic ingredients or prohibited substances (nitrosamines [1], preservatives [2], allergens [3], among others) in cosmetic products are presented.

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**DEVELOPMENT OF NEW BIOIMAGING METHODOLOGIES BY LA-ICP-MS TO STUDY THE ROLE OF ZINC IN AGE-RELATED MACULAR DEGENERATION**

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Nowadays, bioimaging studies are of crucial interest in biomedical research. Besides the importance of quantitative studies, the investigation of trace element distribution along biological tissues could help to understand complex cellular processes. The combination of both approaches can be achieved by using laser ablation (LA) coupled to inductively coupled plasma mass spectrometry (ICP-MS). LA-ICP-MS has been established as a powerful, sensitive and multi-elemental analysis technique that allows obtaining high spatial resolution images of micrometer scale structures. In addition, one of the main advantages of LA-ICP-MS for the analysis of biological samples is that the measurements are carried out directly on the tissue surface preserved in paraffin or in cryogenic conditions. However, cryogenic biological samples allow avoiding any possible metal losses comparing to the preparation processes for paraffin embedding. For such purpose, the use of a cryogenic ablation cell is mandatory in order to keep the integrity of the samples during whole LA-ICP-MS analysis.

The hallmark of Aged-Related Macular Degeneration (AMD), one of the leading causes of irreversible vision loss worldwide, is the formation of extracellular deposits located between the retinal pigment epithelium (RPE) and Bruch's membrane, where the accumulation of Zn can reach millimolar levels. Metallothioneins (MTs) are the main cytosolic proteins that serve as Zn-ion sensors, and are involved in neuroprotection and defense mechanisms against oxidative damage. In previous studies, we proposed the system Zn-MT as a potential therapeutic target in AMD [1].

In this communication we present the analysis of cryogenic ocular tissues and human RPE cells by LA-ICP-MS to obtain Zn-distribution images with high spatial resolution. On the one hand, quantitative images of Zn distribution in cryogenic sections of human ocular tissues, including retina, RPE and sclera will be shown. For quantification purposes matrix-matched standards of gelatin were developed in our laboratory. This methodology was validated by the total quantification of Zn in dissected tissues of RPE, retina and sclera using conventional nebulization ICP-MS after the acidic digestion of the tissues. We carried out complementary analyses of the samples by HPLC-ICP-MS, which allow obtaining the quantitative speciation of Zn, including Zn-MTs, in the water-soluble protein fractions of RPE and neurosensory retina.

On the other hand, preliminary results of a new Zn-bioimaging methodology by LA-ICP-MS in human RPE cells, treated with isotopically-enriched Zn inductors, will be shown. The induction of MTs in RPE cells with two different exogenous Zn reagents have been studied by combining Isotope Pattern Deconvolution (IPD) and LA-ICP-MS technique

[1] González-Iglesias et al, Metallomics 6 (2014) 201-208.

**A NOVEL CALIBRATION STRATEGY FOR LA-ICPMS IMAGING: APPLICATION TO METAL BIOACCUMULATION STUDIES IN ZEBRA FISH LARVAE****M.N. Fernández-Muñiz, J. L. Luque-García, R. Muñoz-Olivas**

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Inductively coupled plasma mass spectrometry (ICPMS) is a sensitive and powerful analytical technique used for the detection of trace elements in a wide variety of samples including biological specimens (e.g., tissue sections, teeth, bones, single hair strands, blood, etc). Laser ablation coupled to ICPMS (LA-ICPMS) allows for direct solid sample analysis and no handling of biological materials is necessary [1]. Nowadays, this technique is becoming the method of choice for trace, ultra trace and isotope analysis of solid samples [2]. The use of LA-ICPMS for quantitative evaluation of biological and medical samples requires suitable quantification procedures [3]. Strategies developed for quantification include: preparation of matrix matched laboratory standards, solution-based calibration, calibration using certified reference materials (CRMs) [4] and film or ink coating of the samples. Preparation of matrix-matched standards, or film coated standards, are time consuming and sometimes difficult to carry out because it is almost impossible to find certified, or very high purity matrix materials.

In this communication, we present a novel quantification strategy for assessing/mapping Ag, Cd and Hg in zebra fish larvae. We developed an alternative calibration method that provides a reliable and easy multielemental semi-quantification. Due to the difficulty in finding matrix-matched standards in LA-ICPMS, we used a fish gelatin as a similar matrix to zebra fish larvae. Moreover, we have tested and evaluated the suitability of an Au solution as a pseudo-internal standard for semi-quantitative imaging experiments. We have validated the approach using fish gelatin standards containing known metal content and, finally, we have applied the proposed approach to zebra fish larvae exposed to the different metals.

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**USO DE TÉCNICAS ORTOGONALES (GC-IMS/CE-UV) PARA CLASIFICAR  
ACEITES DE OLIVA VIRGEN EXTRA**

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Las técnicas ortogonales que suministran información complementaria combinadas con herramientas quimiométricas son estrategias eficientes para resolver problemas analíticos que actualmente no están resueltos si se abordan con una sola técnica.

Por ejemplo, la identificación del régimen de alimentación suministrado al cerdo ibérico o, la diferenciación de un aceite de oliva virgen extra de otros de menor categoría (virgen o lampante), son problemas aún sin resolver por técnicas analíticas individuales. Por ahora, el análisis sensorial es la única herramienta de la que se dispone para clasificar este tipo de alimentos. En el análisis sensorial se detectan los compuestos volátiles con la nariz y se detectan compuestos no volátiles en boca. Por lo tanto, para resolver algunos de los problemas que actualmente solo se pueden abordar con el análisis sensorial es imprescindible la combinación de técnicas analíticas ortogonales.

En este trabajo se ha usado una técnica (Cromatografía de Gases acoplada a la Espectrometría de Movilidad Iónica) para determinar los compuestos volátiles presentes en el aceite de oliva virgen que se detectan en nariz y, otra técnica ortogonal (Electroforesis Capilar acoplada a UV-vis) para detectar los compuestos presentes en el aceite de oliva que el catador aprecia en boca.

Los compuestos detectados por GC-IMS son principalmente aldehídos, alcoholes y cetonas y, los detectados por CE-UV son los compuestos polares (polifenoles entre otros) extraídos del aceite.

En ambos casos, se ha estudiado el potencial de usar toda la información química obtenida en el mapa topográfico o en el electroferograma, frente a la posibilidad de usar marcadores químicos. Una vez seleccionada la mejor estrategia (información global frente específica) se realizará una fusión de los datos obtenidos con ambas técnicas ortogonales para clasificar las muestras de aceite y comparar los resultados con los obtenidos en el análisis sensorial.

**RELATIVE QUANTIFICATION OF COMPONENTS OF MINERAL MIXTURES  
USING ATR-FTIR**

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A method for determining the relative concentration of individual components of mineral samples based on attenuated total reflectance – Fourier transform infrared spectroscopy (ATR-FTIR) is described, including the cases of non-overlapping and overlapping absorption bands. This methodology is a simplification of that based on the use of internal standards for absolute quantification of mineral components in mixtures [1,2]. In this last case, two methodologies, involving respectively the use of absorbance measurements at two and three wavenumbers are described. Such methods were tested for different mixtures of sulfates, carbonates and silicates.

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- [2] F. Bosch-Reig, J.V. Gimeno-Adelantado, F. Bosch-Mossi, A. Doménech-Carbó Quantification of minerals from ATR-FTIR spectra with spectral interferences using the MRC method. Spectrochim. Acta A 2017, 181, 7-12.

**QUANTIFICATION OF MINERALS FROM XRD USING THE CONSTANT RATIO METHOD**

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A method for quantifying the individual components in samples constituted by mixtures of minerals based on X-ray diffraction (XRD) is described. The method, previously applied to ATR-FTIR data [1,2], is based on intensity measurements for analyte peaks relative to the intensity of a selected peak displayed by an internal standard added in a fixed known concentration to the mineral sample. The constant-ratio (CR) method permits the quantification of N analytes using measurements at N fixed diffraction peaks. The method was tested for mixtures of albite, orthoclase, kaolin and quartz.

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# APPLICATION OF RAMAN SPECTROSCOPY FOR DISCRIMINATING MONETARY EMISSIONS: THE CASE OF ANTONINI'S SILVER COINS

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Metal artifacts are among the most common materials in the Cultural Heritage field: alloys have been used in several fields of everyday life and their analysis can prove useful information about the technology of the ancient populations. In particular, coins are an important source or archaeological information, so that the study of their provenance, dating, minting and corrosion processes is of considerable interest [1,2].

In this work a set of Roman silver coins, dated back to the Antonini's period, has been analyzed using Raman spectroscopy aiming to establish the used technique for the silvering and discriminating different mints.

The aim of this work is to investigate the composition and metallographic properties of the alloy with a non-invasive approach. Commonly to investigate the composition of the metal core are used invasive and destructive techniques, therefore the developing of methods without damage on metal is of a great interest.

The study of Antonini's coins is also important as in that historical period Roman Empire underwent to a severe debasement which influenced coinage, so the surface silvering it is still an open question. Diocletian in the 294 A.D. introduced a complex alloy (Cu-Sn-Pb-Ag) with an Ag-rich surface patina of 2 µm [2]. A set of 9 coins from this period was studied. Some coins of the set show a very fine silvered surface with a core composition entirely made of Cu, whereas others have the composition made of Sn-Cu-Pb with a very low concentration of Ag. This technique has been joined with surface analysis (SEM mapping, voltammetry of microparticles (VMP) and electrochemical impedance spectroscopy (EIS)) for a deeper knowledge of the samples. The Raman spectra permitted the identification of Cu<sub>2</sub>O, CuO, PbO, SnO as a main corrosion products. The presence of AgCl has been detected in several coins. Comparison of the Raman profiles permitted to discriminate between coins minted in Rome and coins minted in the Gallia and provided information.

In conclusion, Raman spectroscopy provides information about the composition, structure and thickness of the metal patina which in turn reflects the composition and microstructure of the metal core, ultimately representative of the technology of minting.

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**DEVELOPMENT AND VALIDATION OF A METHOD FOR THE DETERMINATION OF REGULATED FRAGRANCE ALLERGENS BY HPLC-DAD AND PARAFAC2****Jessica Pérez-Outeiral<sup>1</sup>, Saioa Elcoroaristizabal<sup>2</sup>, José Manuel Amigo<sup>3, 4</sup>, Maider Vidal<sup>1</sup>**<sup>1</sup>Department of Applied Chemistry, Faculty of Chemistry, University of the Basque Country (UPV/EHU), P.O. Box 1072, 20080 San Sebastián, Spain<sup>2</sup>Nanoscale Biophotonics Laboratory, School of Chemistry, National University of Ireland, Galway, Ireland<sup>3</sup>Department of Food Science, University of Copenhagen, Rolighedsvej 30 - Frederiksberg C, Copenhagen, Denmark;<sup>4</sup>Department of Fundamental Chemistry, Federal University of Pernambuco, Av. Prof. Moraes Rego, 1235 – Cidade Universitária, Recife, Brazil;  
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Fragrances are important components of daily life products such as perfumes, cosmetics, personal care items, and household and laundry products. Nowadays, there are more than 2500 fragrance ingredients, and although they are generally innocuous, they can sometimes cause skin irritation or allergic reactions. Indeed, currently, there is a list of 24 chemical compounds and 2 natural moss extracts related to fragrances with a well-known potential to cause allergic reactions. These are known in the literature as potentially allergenic substances (PAS), and their presence in cosmetics must be declared on label if their concentration is higher than 0.001% in leave-on products, and 0.01% in rinse-off products [1]. In addition, as PAS are present in all kind of cosmetics, they are easily transferred to water in contact with human body, where their occurrence is notorious. Therefore, there is a great interest in the development of sensitive analytical methods for PAS determination in cosmetics and water samples.

Analytical methods for PAS determination are mainly based on Gas Chromatography-Mass Spectrometry systems commonly coupled to diverse sample pre-treatment techniques. Other techniques have been proposed as alternative. Among them, up to our knowledge, only high performance liquid chromatography with diode array detector (HPLC-DAD) has been successfully applied with a pre-treatment procedure in order to improve the sensitivity of the method [2]. In this work, ultrasound-assisted emulsification microextraction followed by the solidification of the floating organic drop (USAEME-SFOD) to determine 18 out of the 24 PAS by posterior analysis with HPLC-DAD and univariate calibration was applied. The sample pre-treatment allowed decreasing the limits of detection in PAS determination by HPLC-DAD, showing all the advantages of USAEME and SFOD techniques. Nevertheless, the main drawback was the impossibility to determine all the regulated PAS due to high overlapping chromatographic peaks.

In this work, PARAFAC2 has been used with the data obtained with the previous method to determine the remaining 6 PAS: hydroxycitronellal, coumarin, lylal, eugenol, citronellol and farnesol when the other 18 regulated PAS corresponding to well-defined chemical compounds are present. Along the work, the suitability of the selected algorithm for qualitative and quantitative analysis of the analytes is discussed through datasets of increasing complexity. This suitability is assessed by validation of the method performance. Moreover, determination in real samples has been also accomplished.

PARAFAC2 showed to adequately model the data with different instrumental and chemical issues, such as co-elution profiles, overlapping spectra, unknown interfering compounds, retention time shifts and baseline drifts. Satisfactory quality parameters of the model performance were obtained, as well as meaningful chromatographic and spectral profiles. Moreover, low errors of prediction in external validation samples as well as acceptable quantification errors in real spiked samples confirmed the suitability of PARAFAC2 for resolution and quantification of the PAS. The combination of the proposed method with univariate methods for well-resolved peaks allows the determination of the 24 regulated PAS by HPLC-DAD.

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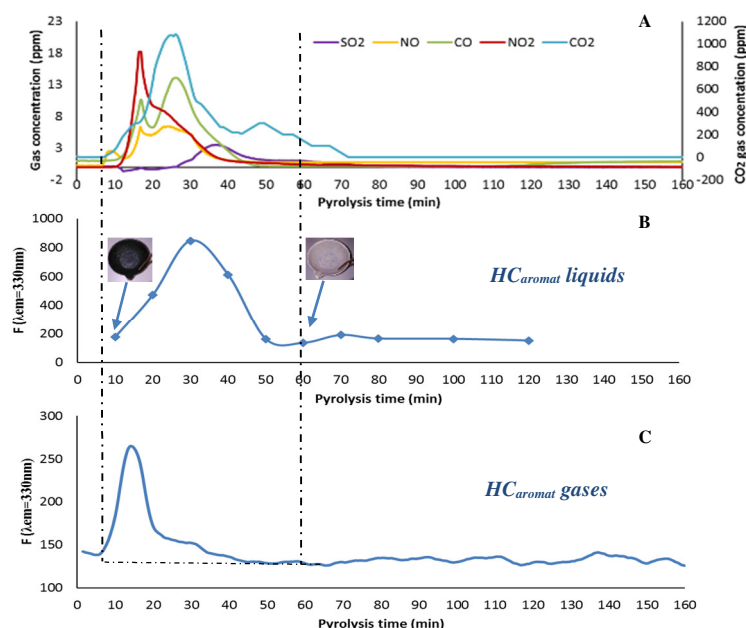
## GAS-PHASE FLUORESCENCE FOR MONITORING ORGANIC COMPOUNDS GENERATED DURING HOUSEHOLD OVENS PYROLYSIS

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Pyrolytic or self-cleaning ovens use high temperatures, around 500°C, to break down the remained by-products of baking. Organic matter decomposes in volatile compounds of low molecular weight and water, while the inorganic matter is converted to ash, which can be easily removed with a damp cloth. Current ovens do not usually have smart control of the pyrolysis so high-energy consumption is involved. Gas sensors for CO<sub>x</sub>, NO<sub>x</sub> or SO<sub>x</sub> could be used for this aim, however they do not give a real concentration of organic matter remaining in the oven. The aim of this work is to know if fluorescence could help to monitor this process

To do this, a household oven is stained with a standard mixture resembling the remaining dirty coming from a conventional baking process (coconut oil and meat concentrate). Then, the mixture was baked for 3h at 250°C, and later subjected to a pyrolysis process during 2h. To monitor this process, it has been designed a sampling procedure, based on the continuous pumping of the pyrolysis gases generated inside the oven into a chamber which contains a gas sensor platform (NO, NO<sub>2</sub>, CO, CO<sub>2</sub> and SO<sub>2</sub>). In this way, it has been verified that the formation of these gases begins to increase in the first 10 min and that, after about 50 min begins to decrease until the initial value (Fig.1A). This time coincides with the formation of the ashes, indicating the moment in which all the organic matter decomposes and the cleaning process is completed.



**Figure 1**

**A)** Gas concentration vs pyrolysis time during continuous monitoring in the pyrolysis process.

**B)** Fluorescence intensity of the maximum emission (330 nm) of organic liquid compounds in function of pyrolysis time (λ<sub>ext</sub>=224 nm)

**C)** Fluorescence intensity of the maximum emission (330 nm) of HC gases in function of pyrolysis time (λ<sub>ext</sub>=224 nm).

The fluorescence optical properties of the baked samples (dissolved in hexane) have been studied at different pyrolysis times, after samples extraction in hexane. A fluorescence signal (λ<sub>ext</sub>=224nm and λ<sub>em</sub>=330nm), running in parallel with the profile of gases observed through the sensing platform was observed. With this premise, the fluorescence of the gases (probably HC<sub>aromat</sub>) generated during the pyrolysis was also monitored by pumping the gases at the exit of the oven to a flow through cell placed in a fluorimeter. The fluorescence spectra of these HC gases (Fig.1C) were quite similar to those obtained for HC liquids.

These result suggest that, gas phase fluorescence variations of HC<sub>aromat</sub> can be considered as a candidate to monitor the pyrolysis process.

This work has been supported by BSH-Spain within the project SMART OVENS III.

## SURFACE WETTABILITY SETTING. INFLUENCE ON BIOASSAY PERFORMANCE

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In biosensing, it is important to modulate the support properties, having into account the features of the biointeraction to be employed. Wettability is a variable that can help to improve the performances of the analyte binding/recognition. This property, determined by measuring water contact angle (WCA), can be modified in several ways; in this work the chemical surface modification of a common support (glass) has been applied with this goal. Different organosilane approaches for surface derivatization have been studied: terminal hydrocarbon chain ended in alkene with different number of carbon atoms (from C02 to C22), alkene-ended silanes substituted with fluorine atoms (Figure 1, grey bars and full dots), the same ones modified with 1% of a fluorinated alkyl silane (Figure 1, yellow bars and hollow triangles), and mixtures of short(C04)-large(C22) hydrocarbon chain silanes (Figure 2). We take advantage of the C=C double bond for the covalent array anchoring of thiolated biotin by means of the photochemical *click* thiol-ene reaction [1], and the further binding of Cy5-labelled streptavidin allows displaying the binding events and viewing the influence of wettability on signal performance (intensity, SNR).

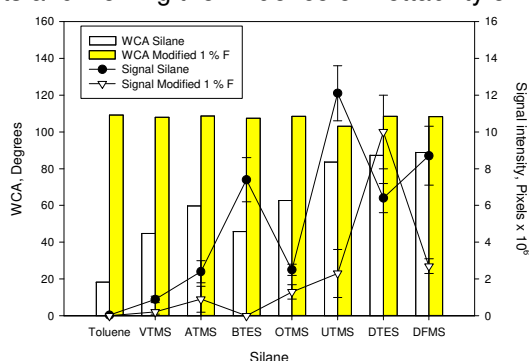


Fig. 1. WCA and signal intensity achieved treating glass with alkenyl silanes pure and modified with 1% fluorinated alkyl one

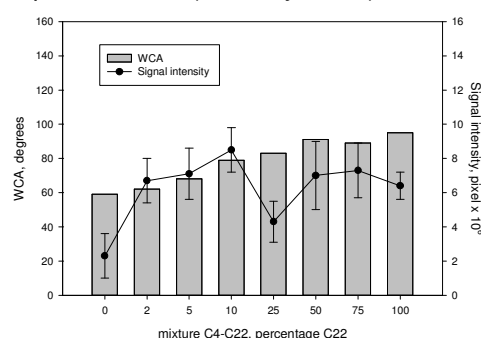


Figure 2. WCA and signal intensity achieved silanizing glass with mixtures C04-C22 alkenyl silanes

Results showed that mild hydrophobic surfaces (90-100°), achieved with large and fluorinated chain silanes produced best results in terms of lowest unspecific binding and highest signal intensity. Modification 1% fluorinated silane achieved surfaces with the same hydrophobicity (WCA 110-120°) and improved SNR, even silanes as short as C2 and C3. Binary mixtures of C04 and C22 silanes allowed modulating wettability, in a useful range, with WCA values between 50 and 100 degrees. Also, achieved spots were big and low intense ones for 100% C04, but for low C22 percentages (10 and even 5%), low-sized and more intense signals are recorded, allowing the identification of biotin concentrations as low as 0.04 mM.

As conclusion, glass derivatization with the appropriate silane, or better silane mixtures, allows modulating the surface wettability with the aim of improve binding bioassays. For biotin-avidin system, mild hydrophobicity has shown to be best, with large and fluorinated silanes, or hydrophobic-hydrophilic silane combinations.

#### Acknowledgement

Financial support from the Generalitat Valenciana (PROMETEO/2014/040), FEDER and the Spanish Ministry of Economy and Competitiveness (CTQ2013-45875-R) is acknowledged.

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## APPLICATION OF A PERFLUORINATED SURFACE TO THE DISCRIMINATION OF SINGLE NUCLEOTIDE POLYMORPHISMS AND BACTERIAL DNA DETECTION

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Microarray technology is an interesting option to carry out the detection of multiple targets in a fast, easy and inexpensive way [1]. The surface nature of the supports to be used is key to reach the demanded performances. To accomplish this challenge, improved surfaces and immobilization strategies are key to obtain outstanding materials.

In this communication, a reaction for the attachment of different receptors onto highly hydrophobic surfaces, was studied. This novel light induced reaction allows the immobilization of thiolated DNA probes to perfluorinated surfaces via fluor-thiol coupling chemistry.

Sensitivity studies were performed to make sure the suitability of these platforms to detect oligonucleotide sequences based on hybridization tests, reaching a sensitivity of 0.5 nM.

Taking into account the good quality of the perfluorinated surface, the substrates were applied to the discrimination of single nucleotide polymorphisms (SNPs), important in the study of disease susceptibility and response to drugs [2]. Then, probes with different complementarity were patterned onto the support. Using optimal stringency conditions, (25 % of formamide and SSC0.1x) clear discrimination was achieved, with up to 85% of fluorescence signal increase for the fully complementary strand concerning to one mismatch sequence.

Finally, to extend the applicability of these surfaces, amplicon bacteria detection was performed. A probe complementary to the central region of a 152 bp *Salmonella* amplicon was used and up to 1.7 pM of *Salmonella* PCR products were detected selectively.

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### Acknowledgements

Financial support from INTERBOINTER (project CTQ2013-45875-R) and BIHOLOG (project CTQ2016-75749-R), FEDER and GVA PROMETEO II 2014/040 is acknowledged. P. J.-M. acknowledges to Spanish Ministry of Economy, Industry and Competitiveness for the public FPI grant (Project CTQ2013-45875-R) and the co-financing by the European Social Fund.

## OPTIMIZATION OF AN ORBITRAP HIGH RESOLUTION MASS SPECTROMETER FOR RETROSPECTIVE SCREENING OF ENVIRONMENTAL CONTAMINANTS IN URINE

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The ever growing number of chemicals being used, such as pesticides, care products, UV filters, parabens and so on, has an impact on the environment and therefore on humans [1]. In human biomonitoring, biomarkers of exposure to these chemicals are determined in biological samples such as urine in order to evaluate the internal exposure. These chemicals are present at concentrations of few ng·mL<sup>-1</sup> and consequently biomarker determination requires sensitive and selective analytical methods. The usual analytical methodology for polar biomarkers in biomonitoring studies is the liquid chromatography coupled to tandem mass spectrometry [2]. However, the introduction of the high-resolution (>50,000 FWHM) mass-accurate (<5 ppm) spectrometers, such as Q-TOF or Orbitrap, have allowed the implementation of combined quantitative target and post-run target analytical strategies for comprehensive determination of pesticides and other emerging contaminant biomarkers.

In this work, an ultra high performance liquid chromatography coupled to Orbitrap high resolution mass spectrometry (UHPLC-HRMS) was used. The main parameters governing the mass accuracy and fragmentation of the mass spectrometer were optimized for the determination of pesticide biomarkers in urine. We optimized the Resolving Powers (10,000; 25,000 and 50,000 FWHM), the fragmentation mode (Collision Induced Dissociation and High-energy Collision Dissociation) and compared internal and external calibration.

Subsequently, a post-target methodology for the detection of environmental contaminant biomarkers in human urine was developed. For the retrospective analysis, a theoretical database of 102 environmental pollutant biomarkers including their isotopes and fragments was built up using previous literature [3] and databases [4]. The retrospective screening methodology was applied to 50 human urine samples.

The best results were achieved with a Resolving Power of 25,000 and by applying Collision Induced Dissociation fragmentation mode (40eV). The analytical response was improved when internal calibration with caffeine was used. The screening retrospective methodology tentatively identified 8 contaminant biomarkers, 4 out of which (Monobenzyl phthalate, Mono-2-ethyl-5-carboxypentyl phthalate, 4-(N,N-dimethylamino)benzoic acid and Triclosan) were confirmed using analytical standards.

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## DEVELOPMENT OF A METHOD FOR THE ANALYSIS OF PARABENS AND BISPHENOL A IN HUMAN MILK BY USING DISPERSIVE SOLID-PHASE EXTRACTION CLEAN-UP AND LC-MS-MS

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The human risk assessment of exposure to chemicals traditionally follows the classical approach of estimating the external exposure (food, air, water), and comparing it with health-based monitoring guidance values (HBGVs) [1]. However, there is a growing interest in evaluating exposures to environmental chemicals using human biomonitoring (HBM).

Synthetic chemicals such as bisphenol A (BPA) or parabens (PBs) are common endocrine disrupting chemicals (EDCs). The main routes of human exposure to these compounds are dermal contact, ingestion or inhalation [2,3]. The assessment of exposure to EDCs is especially important in the case of breastfed infants, who are in the first stages of development being therefore more vulnerable and susceptible to changes in the endocrine system. Human breast milk has been proposed for the assessment of exposure to environmental chemicals [4], particularly to EDCs because it is possibly the main route of exposure for babies. For this reason, the development of analytical methods for determination of EDCs at trace levels in this matrix becomes of great importance.

A sensitive, accurate, rapid and easy method has been proposed, optimized and validated for quantitative determination at trace level of methyl-, ethyl-, propyl- and butylparaben and bisphenol A in human milk samples. The method includes acetonitrile extraction, fat precipitation by cooling pre clean-up followed by dispersive solid-phase extraction (d-SPE) with C18 sorbents and PSA based on QuEChERS procedure clean-up. Different combinations of d-SPE extraction reagents and sample amounts were tested in order to minimize matrix co-extractives and interferences. Best recoveries were obtained with 1200 mg of MgSO<sub>4</sub>(4), 400 mg of end-capped C(18), 400 mg of PSA and 1 g of sample amount. Determination was performed by liquid chromatography-tandem mass spectrometry (LC-MS/MS) analysis. Atmospheric pressure chemical ionization (APCI) in the selected reaction monitoring (SRM) mode was used for MS detection in order to increase the BPA response. The use of two reactions for each compound allowed simultaneous quantification and identification in one run. The analytes were separated in less than 10min. Deuterium-labeled ethylparaben-d<sub>5</sub> (EPB-d<sub>5</sub>), and deuterium-labeled bisphenol A-d<sub>16</sub> (BPA-d<sub>16</sub>) were used as surrogates.

The limits of quantification is 0.1 ng mL<sup>-1</sup> for all the compounds, while inter- and intra-day variability was under 9% in all cases. In the absence of certified reference materials, recovery assays with spiked samples using matrix-matched calibration were used to validate the method. Recovery rates ranged from 82% to 107%. The proposed method was satisfactorily applied for the determination of four selected parabens and bisphenol A in human milk samples obtained from nursing mothers living in the province of Valencia (Spain).

Results of this study showed that this technique is applicable in routine analysis for its application into monitoring programs. It simplifies time-consuming clean-up steps and allows a satisfactory long-term chromatographic performance.

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## TRANSFERENCE OF OPTIMAL SEPARATIONS IN MULTI-LINEAR GRADIENT ELUTION TO AGED SERIALY-COUPLED COLUMNS

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Single conventional columns in reversed-phase liquid chromatography are insufficient for analysing complex samples, due to their limited functionality. An interesting possibility for increasing the separation power is the combination of several columns with different stationary phases. Thus, by fine tuning the coupled segments, the selectivity of the tandem column can be adapted to the separation requirements of a particular sample. Furthermore: each combination (column nature, length and order of the participant segments) behaves as a new hybrid column with new separation features, often outperforming the results offered by the individual columns. A full exploitation of this approach requires, however, the development of powerful interpretive optimisation strategies, able to scan efficiently the possibilities of the separation system. The most useful search configuration requires not only finding out the optimal combination of coupled segments, but also the profile of the gradient program flushing through it, which should preferably be multi-linear.

With the routine use, chromatographic columns inevitably lose some separation performance, which is translated in shifts in retention to smaller times, and in a certain loss in column efficiency as well. These losses are in some instances hard to avoid, such as in conventional columns intensively used along months, in columns operating under stressing conditions (e.g., extreme pH values or high temperatures in the mobile phase), or when short columns are used in fast separations of biological compounds in clinical analysis. Another case is precisely serially coupled columns. Indeed, when column segments are hyphenated to build a hybrid system, the combined backpressure may easily exceed the recommended operative levels for the first segments in the assembly, and this contributes to column ageing. As a result of the repeated usage, the inner structure of the packing may gradually change. The theoretical plate count is a good indicator on how well a given column is performing. As long as the deterioration in efficiency is not excessive, the column can still offer interesting separations, which may be sufficient for our analytical purposes. This means that the column can be used over a longer time, which decreasing analysis cost, but periodic monitoring is required over the column lifetime.

In this work, a method developed at the beginning of the column lifetime, which offered a good separation performance, is adapted after years of intensive use and considerable ageing, which implies altering the models initially developed to adapt the new system performance, parametrizing the ageing. For this purpose, a mixture of 15 sulphonamides was used. Three types of stationary phases were examined (C18, phenyl and cyano), both individually and pairwise connected in series as well. The length was constant, being 5.0, 5.0 and 7.5 cm for the three mentioned columns, respectively. Good resolution could be found by parametrising the ageing, which allowed correcting the models developed before the deterioration and adapt the optimal gradients. An excellent correlation was found between predicted and experimental chromatograms.

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## FOURIER TRANSFORM INFRARED ANALYSIS OF COMMERCIAL FORMULATIONS FOR VARROA TREATMENT

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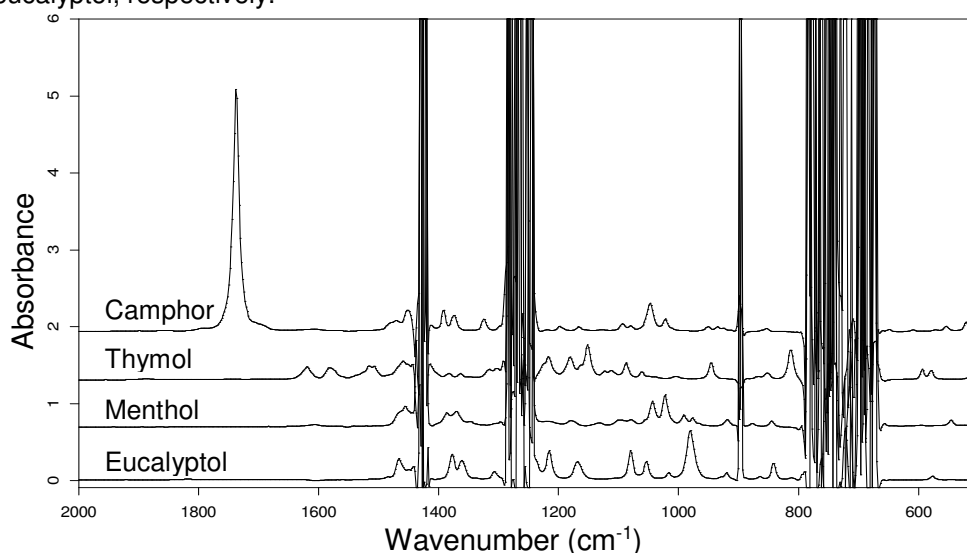
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Ecoparasitic mite, *Varroa destructor*, is considered as the most serious worldwide pest of western honey bee. The aim of the present work was to compare the use of univariate and multivariate calibration for the rapid simultaneous determination of four monoterpenes in commercial formulations used for varroa treatment, by means of FT-IR using mixture of analyte standards.

Simultaneous determination of camphor, thymol, menthol and eucalyptol in commercial formulations used for varroa treatment was made. Absorbance peak heights of transmission middle infrared (MIR) spectra of individual monoterpenes, prepared in dichloromethane, were measured at 1737, 1151, 1022 and 980 cm<sup>-1</sup> (corrected with a base-line at 1933 cm<sup>-1</sup>) for camphor, thymol, menthol and eucalyptol, respectively. Data were processed using the proportional equations approach in univariate mode. For multivariate calibration, partial least squares (PLS) regression, based on a classical 4<sup>2</sup> design for standards, was employed, using the information from spectral ranges between 1812-1667 cm<sup>-1</sup> and 1200-955 cm<sup>-1</sup> to build PLS models considering different spectra pre-processing after mean centering of infrared data. The root mean square error of prediction (RMSEP) was considered to select the best model for each monoterpene. Accuracy of both assayed strategies was evaluated from samples spiked with different amounts of the four compounds. Recovery percentages from 91% to 94%, 105.5% to 116.9%, 95% to 108.2% and from 100.1% to 107.1% were achieved for camphor, thymol, menthol and eucalyptol, respectively.



**Figure:** FT-IR spectra of camphor, thymol, menthol and eucalyptol standards, diluted in dichloromethane at a concentration of 5 mg mL<sup>-1</sup>.

### Acknowledgements.

Authors acknowledge the financial support of Ministerio de Economía y Competitividad-Feder (Project CTQ 2014-52841-P) and Generalitat Valenciana (Project PROMETEO II/2014/077).

**ELECTROCHEMICAL IMPEDANCE SPECTROSCOPY FOR CHARACTERIZATION OF SURFACE MONOLITHS PREPARED IN CAPILLARY SYSTEMS**

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The development of monolithic columns in miniaturized separation techniques such as capillary/nano liquid chromatography and capillary electrochromatography has generated considerable interest in the last years. Morphological characterization of these materials is an important issue in order to understand its chromatographic properties. In particular, the surface area has been commonly measured by traditional techniques such as nitrogen adsorption-desorption isotherms. However, this methodology requires quite large samples (ten of milligrams) per analysis, amounts that can hardly be recovered for surface area characterization from materials prepared in capillary format. To overcome this problem, the common practice adopted has been prepared in parallel bulk-phase synthesis using the same polymerization mixture and conditions as in the preparation of capillary monoliths. However, this scale-up approach monolith synthesis is rather questionable, since the diameter of the mold has a substantial influence on the surface area [1, 2]. In this sense, a procedure capable of measuring the surface area or surface roughness in capillary monoliths would be highly desirable.

Electrical impedance spectroscopy (EIS) is a simple non-destructive and non-invasive technique to characterize membranes and monitor membrane processes [3-5]. The electrical impedance is the complex electrical resistance that is defined by the ratio of an alternating voltage to its corresponding current as well as the phase difference between them. The promise of EIS is that, with a single experimental procedure covering a sufficiently broad range of frequencies, the influence of the governing physical and chemical phenomena may be isolated and distinguished at a given applied potential. Thus, electrical characteristics of membranes that can directly be determined by EIS measurements are the electrical conductance, capacitance and inductance. Additionally, physical properties (porosity, thicknesses or surface roughness) can be derived out of EIS spectra indirectly by creating models in the form of equivalent circuits [3-6].

In this work, the application of EIS methodology was applied to the measurement of the surface roughness of monoliths in 100  $\mu\text{m}$  i.d. capillaries prepared using thermal and UV initiation. The influence of composition of polymerization mixture (i.e. different ratios of monomer/crosslinker and porogenic solvent mixture) on morphological properties of monolithic columns was investigated. Also, the effect of the addition of magnetic nanoparticles to the polymerization mixture was examined in order to detect changes in the surface roughness of hybrid monoliths. The results showed a good correlation between the EIS measurements (surface roughness) of the materials and those obtained by the parallel bulk polymerization data.

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**SPECTROSCOPIC MULTIVARIATE KINETIC ANALYSIS OF SIZE AND CONCENTRATION OF SILVER NANOPARTICLES. APPLICATION TO WATER SAMPLES**

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The application of nanomaterials has increased in the last two decades and their presence in a great variety of products (textiles, cosmetics, electronics...) [1] could lead them to reach waste water treatment plants (WWTPs) where they can escape elimination and enter in the aquatic environments and drinking water. Among nanoparticles (NPs), silver nanoparticles (AgNPs) have attracted especial attention because of their antibacterial properties favoring their presence in medical products, gels, deodorants, air and water filters... [2]. One of the main limitations in assessing NPs impact is the lack of reliable methods for the detection and quantification of NPs in complex systems. There is a current trend of using multi-method approaches including light scattering measurements, classical electron microscopy and analytical separation techniques. Unfortunately, this methodology is not affordable for many quality control laboratories.

Based on the dependency of plasmon resonance (SPR) absorption band of NPs with size, and consequently, with the formation of aggregate / assembled structures [3], in this work the variation of the spectroscopic properties of the AgNPs has been used to simultaneously determine their concentration and their size. To reach this objective, the effect of time, temperature, pH and aggregating substance on the formation of stable aggregates of citrate- AgNPs was first evaluated. As it was a kinetic process, both initial and final values of temperature and time were first fixed to record the changes in the absorption spectra. Subsequently, multivariate approaches and experimental design based on response surface methodologies (RSA) were employed with the aim of modelling analytical responses and setting optimal factors combination. In this line, a factorial experimental design was developed to optimize the type and concentration of buffer and aggregating compound. Spectra obtained using AgNPs of different sizes (20, 40, 60 and 80 nm) at different concentration levels (between 0.633 and 2.5 mg l<sup>-1</sup>) allow us to build a multivariate calibration based on a partial least squares model (PLS). Principal component analysis (PCA) was applied to select the range of wavelengths as well as the number of factor for the PLS calibration model. Regarding calibration mixtures there are practical and economic limitations on the number of calibration samples to be employed, in the current work a design using 21 mixtures (Simplex Lattice Design plus three replicates in the centroid) was developed for ensuring that all important variations are been included. Prediction Error Sum of Squares, PRESS, was used to select the appropriate number of latent factors with predictive capacity. Nine latent factors were used to validate the model using nine synthetic mixtures. Finally, the developed method was applied to water samples of different nature (drinking water, wastewater and river water) doped with mixtures of AgNPs of different size and concentration levels.

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**DETERMINACIÓN DE BENZO(A)PIRENO EN AEROSOL ATMOSFÉRICO MEDIANTE EXTRACCIÓN ASISTIDA POR MICROONDAS Y DETECCIÓN POR HPLC-FL**

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El benzo(a)pireno (BaP) es un agente carcinogénico originado principalmente en reacciones de combustión, cuya concentración en el aire ambiente está regulada por normativas de protección de la salud humana. Así, la directiva europea 2004/107/CE, relativa al arsénico, el cadmio, el mercurio, el níquel y los hidrocarburos aromáticos policíclicos en el aire ambiente, estipula un objetivo de concentración de benzo(a)pireno de  $1 \text{ ng/m}^3$  como promedio anual, analizado en la fracción PM<sub>10</sub> del aerosol atmosférico. Debido a la complejidad de la matriz y la baja concentración de BaP en las muestras de aerosoles ambientales, el pretratamiento de las muestras es un factor decisivo en las metodologías analíticas desarrolladas para su determinación. Se exploran desde hace unos años diversos tipos de pretratamiento alternativos a los tradicionales basados en la extracción Soxhlet, entre los cuales se encuentra la extracción asistida por microondas. En esta comunicación, presentamos los resultados del desarrollo de una metodología de análisis de BaP de muestras de aerosol PM<sub>10</sub> capturado sobre filtros de fibra de cuarzo mediante captadores de alto volumen. La metodología consiste en la extracción por microondas del BaP depositado en el filtro (medio acetona:hexano 1:1), seguida de una preconcentración en corriente de nitrógeno, recuperación del residuo con acetonitrilo y detección de BaP por HPLC con detección fluorescente. Se obtuvo un límite de detección de  $1.0 \times 10^{-3} \text{ ng/m}^3$ , adecuado para la determinación del analito en muestras procedentes de lugares poco contaminados. El aseguramiento de calidad se efectuó mediante el análisis del material de referencia certificado ERM®-CZ100 "PAH in Fine Dust (PM<sub>10</sub>-like)", obteniéndose una recuperación media del 86%, con una desviación estándar relativa del 3.6%. La metodología optimizada se aplicó a la medida de la concentración de BaP en 115 muestras de la red de vigilancia de la calidad del aire de Extremadura durante un período de un año. Se investigó la variabilidad espacial y temporal del BaP en el territorio cubierto por la red, así como la influencia de las condiciones meteorológicas y de los niveles de otros contaminantes atmosféricos. Los niveles de BaP medidos están en el rango de variabilidad típico de entornos rurales según datos de la red EMEP, siendo en todo caso muy inferiores a los valores de protección marcados por la legislación. Se encontró una variación espacial poco significativa pero sí una marcada evolución anual con valores de concentración más elevados en los meses fríos, cuando el BaP se encuentra mayoritariamente ligado al aerosol respecto a la fase gaseosa. Se aplicó un procedimiento normalizado para estimar el riesgo de cáncer de pulmón causado por la inhalación de BaP para los habitantes de las zonas analizadas, encontrándose un riesgo medio de 8 casos por millón de habitantes en adultos, mientras que en niños menores de 2 años el riesgo es hasta 10 veces superior.

**Agradecimientos:** Al MEyC (CTQ2014-52309-P) y a la Junta de Extremadura (Ayuda GR15087), ambos co-financiados mediante fondos europeos FEDER. A la red de vigilancia de la calidad del aire de Extremadura (red REPICA), por el suministro de muestras y la financiación del trabajo mediante un convenio de colaboración con la UEx.

# SCREENING OF ESTROGENIC COMPOUNDS IN CONSUMER-ELECTRONICS PLASTICS BY LIQUID CHROMATOGRAPHY NANOFRACTIONATION-BIOACTIVITY DETECTION AND MASS SPECTROMETRY

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The chemical safety of consumer products is an issue of emerging concern. Plastics are widely used, e.g. as casings of consumer electronics (TVs, computers, routers, etc.), which are present in houses and offices in continuously increasing numbers and are known to contain a variety of chemical additives. Plastic leachates have been reported to exert aquatic toxicity which varied with the type of plastic and the weathering conditions.<sup>1,2</sup> More specifically, estrogenic activity has been reported in leachates from polycarbonate and epoxy resins used as food contact materials that are known to contain bisphenol A (BPA) as an unpolymerized residue.<sup>3,4</sup> More recently, also plastic leachates made up of other polymers (the so-called BPA-free plastics) have been reported to exert estrogenic activity too, although individual chemicals were not identified.<sup>5,6</sup> A recent study of our research group reported that plastic casings of electronic equipment contain a variety of phthalates, UV filters, antioxidants, flame retardants and related compounds.<sup>7</sup> Among these chemicals, some phthalates (e.g. butyl benzyl phthalate, dibutyl phthalate), antioxidants (e.g. 2-hydroxy-4-methoxybenzophenone, 2,2'-dihydroxy-4-methoxybenzophenone) and flame retardants (e.g. triphenyl phosphite) have been reported to be estrogenic.

In this study, we investigated the estrogenic activity of components of plastics coming from electronics' casings. A recently developed fractionation platform for effect-directed analysis (EDA) was used.<sup>8</sup> The platform combines liquid chromatography (LC) with high resolution time-of-flight mass spectrometry (TOFMS) in parallel with a human cell (VM7Luc4E2) gene reporter assay via nanofractionation for the detection of estrogenic compounds. The platform allows reconstruction of bioassay chromatograms that can directly be correlated to the parallel obtained MS chromatograms, allowing straightforward pinpointing of accurate masses of estrogenic compounds. Results were obtained within a single fractionation cycle, resulting in a drastic decrease of total analysis time, and thus increase in throughput compared to traditional EDA studies. Four out of eight of the analysed plastics samples showed presence of estrogenic compounds. Based on the MS results these were assigned to bisphenol A (BPA), 2,4-di-tert-butylphenol and a possible bisphenol A analog. Flame retardants that were present in the analyzed samples, did not show any estrogenic response in the human cell-based bioassay. However, bisphenol A and the suspected analog could be present as an impurity of the flame retardants BDP, TBBPA and TBBPA-based polymers. In general, we could conclude that plastic casings from consumer electronics contained estrogenic compounds. Consequently, these common consumer products could constitute a source of estrogenic contamination for human exposure indoors but also at primitive electronic waste recycling sites.

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# DETERMINATION OF NATURAL AND SYNTHETIC HORMONES IN WATER USING A CONTINUOUS SOLID-PHASE EXTRACTION AND GAS CHROMATOGRAPHY–MASS SPECTROMETRY

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Hormone monitorization in the aquatic environment has progressively become a priority for governments and regulatory agencies as well as for the population. Estrogenic, progestogenic and androgenic hormones are a figure of concern due to their wide occurrence and high risk to humans and wildlife. They are produced naturally in the mammal body, in industry, they are synthetically created for its use in birth control pills and hormone replacement therapies, because of their heavy use by people daily they are introduced in the aquatic environment via the effluents of wastewater treatment plants (WWTP) and from agricultural runoffs. Several studies have reported that some hormones can affect the reproductive physiology and behavior of many fish species, particularly in ecosystems receiving high levels of poorly diluted WWTP effluents [1]. Certain hormones (estrone, diethylstilbestrol, 17 $\beta$ -estradiol, 17 $\alpha$ -ethinyloestradiol, estriol, hexestrol, testosterone, progesterone, norethindrone and levorgestrel) had been included as priority or emerging pollutants in the European Water Framework Directive, for that, the development of analytical methodologies for the isolation and determination of those steroid hormones are needed [2].

In literature, several methodologies use different chromatographic techniques for the determination of hormones in water such as liquid chromatography–mass spectrometer (LC/MS), liquid chromatography-tandem mass spectrometer (LC-MS/MS), high-performance liquid chromatography–diode array detection (HPLC-DAD), gas chromatography-mass spectrometer (GC-MS) and gas chromatography-tandem mass spectrometer (GC-MS-MS). The isolation of those contaminants is generally based on different techniques, such as liquid–liquid extraction (LLE), solid–phase extraction (SPE), dispersive liquid–liquid microextraction (DLLME), solid-phase microextraction (SPME) or stir bar sorptive extraction (SBSE). SPE is one of the most preferred choice, thanks to its polyvalence to multi-residue analysis of compounds, spanning a wide range of polarity or possessing diverse physic-chemical properties. The SPE technique is usually implemented by using a small column or cartridge containing an appropriate sorbent, for example, polymers with hydrophilic-lipophilic balance, C18 cartridges or polymeric sorbents, graphitized non-porous carbon sorbent or, in recent years, molecularly imprinted polymers [3].

A novel analytical method, using a continuous solid-phase extraction (SPE) system in combination with gas chromatography–mass spectrometry, has been developed for the simultaneous isolation and determination of hormones in the aquatic environment. Samples were preconcentrated by using an automatic solid-phase extraction module containing a sorbent column, after elution, the analytes are derivatized with a mixture of N,O-bis(trimethylsilyl)trifluoroacetamide and trimethylchlorosilane making them suitable to the determination by GC techniques. The proposed method was validated with good analytical properties, including very short chromatographic run time of 15 min, acceptable recovery values, good linearity throughout the studied concentration ranges and good precision (relative standard deviations less than 7%) for the determination of hormones in drinking, river, pond, well, swimming pool and waste water samples.

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## HIGH RESOLUTION MASS SPECTROMETRY FOR ENVIRONMENTAL WATER MONITORING

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The presence of organic pollutants in aquatic ecosystems is a potential risk for the environment and human health. In view of this, the European Union (EU) introduced the Water Framework Directive (WFD), thereby establishing guidelines to control the pollution of surface water, indicating a list of priority substances and setting out environmental quality standards (EQS) [1] for these pollutants.

After an extraction step, gas chromatography (GC) followed by mass spectrometry (MS) or tandem mass spectrometry (MS/MS) are the most commonly used techniques for the determination of organic pollutants. Nevertheless, the low EQS values established for most of the pollutants in water still require the development of highly sensitive and selective methods capable of simultaneously determining a broad range of compounds possessing different chemical properties at ultra-trace levels. In this respect, the application of high resolution mass spectrometry (HRMS) can be considered as a potential tool for the analysis of pollutants in environmental samples due to its high sensitivity and specificity.

In the present study, a methodology based on the on-line combination of headspace solid phase microextraction (HS-SPME) and gas chromatography coupled to a magnetic sector double-focusing high resolution mass spectrometer (GC-HRMS) for the fully automated determination of priority substances in environmental water samples is proposed. Indeed, the method involves the accurate monitoring of pesticides, polyaromatic hydrocarbons (PAHs) and polybrominated diphenyl ethers (PBDEs), listed as priority substances, and even the highly persistent polychlorinated biphenyl congeners (PCBs) which are currently under revision by the EU.

HS-SPME as well as GC-HRMS conditions were optimized so as to achieve maximum extraction efficiency and sensitivity which was reinforced by using multiple ion detection (MID) as acquisition mode.

Method validation showed good linearity ( $R^2 > 0.99$ ), recoveries between 86 and 113%, relative standard deviation (RSD) values  $< 20\%$  for intra-day and inter-day precision and quantification limits (LOQs) between 0.01-350 ng L<sup>-1</sup>. Finally, the proposed method was successfully applied to surface waters as well as wastewater samples collected in Andalusia (South of Spain), fundamentally revealing the presence of certain pesticides (chlorpyrifos, p,p'-DDD and p,p'-DDT and the metabolite p,p'-DDE) and anthracene in some of the analyzed samples.

The achieved results show that the proposed methodology is reliable, reproducible, and robust allowing for rapid and accurate multiclass screening analysis and at the same time a cost- and time-effective monitoring tool. Therefore, its use in research and routine analysis laboratories would be a great asset.

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*The authors gratefully acknowledge Andalusian Regional Government (Regional Ministry of Innovation, Science, and Enterprise) and FEDER for financial support (Project Ref. P-12-FQM 1838). ID is also grateful for personal funding through the same Project Reference.*



## DETERMINACIÓN DE OLORES EN AGUAS MEDIANTE HS-SPME-GC-MS

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La presencia a niveles traza de determinados compuestos que imparten al agua olores y sabores desagradables afecta de forma importante a su calidad para consumo. El umbral de percepción de alguno de estos compuestos se encuentra en niveles de partes por trillón. Por ejemplo, la geosmina (GSM) y el 2-metilisoborneol (MIB), producidos por cianobacterias, son responsables del típico olor a tierra y humedad, con umbrales entre 1 y 10 ng/l<sup>[1]</sup>. Otros episodios de olor y sabor en agua han tenido su origen en vertidos de plantas químicas productoras de resinas<sup>[2-3]</sup>. Los compuestos responsables identificados en estos casos fueron el 2-etil-5,5-dimetil-1,3-dioxano (EDD)<sup>[2]</sup> y el 2-etil-4-metil-1,3-dioxolano (EMD)<sup>[3]</sup> que imparten un olor dulce nauseabundo y cuyos umbrales están entre 5 y 10 ng/l<sup>[3-4]</sup>.

En esta comunicación se presentan los resultados obtenidos en el desarrollo y validación de un método para la determinación de estos cuatro compuestos en muestras de agua. El método se basa en una extracción en fase sólida del espacio de cabeza de las muestras en línea con una cromatografía de gases acoplada a espectrometría de masas. Aunque esta técnica ha sido descrita para la determinación de GSM y MIB<sup>[4-5]</sup>, y es usada en el método ISO 17943 para la determinación de 61 VOCs en aguas, no se ha descrito el método para EDD y EMD en la literatura. Además, es la primera vez que se optimiza para estos cuatro compuestos de forma conjunta. El objetivo final de este trabajo es desarrollar un método sensible que permita evaluar la eficacia de distintos catalizadores plasmónicos en su fotodegradación con luz solar en estaciones depuradoras de aguas.

En primer lugar, se estudió la influencia de la fibra y la adición de sal. Los mejores resultados para EMD y EDD se obtuvieron con la fibra triple DVB/CAR/PDMS-, mientras que la fibra PDMS/DVB era mejor para la extracción de GSM. En todos los casos la adición de NaCl aumentó el rendimiento de la extracción. A continuación, se optimizaron las condiciones de temperatura y volumen de muestra mediante un diseño central compuesto y la metodología de superficies de respuesta. Las condiciones óptimas encontradas con la función de conveniencia obtenida a partir de las funciones individuales de los cuatro analitos fueron 45°C y 4,5 ml de muestra. Por último, 15 min de extracción fueron suficientes para alcanzar el equilibrio.

La cuantificación de los compuestos se realizó en modo SIS seleccionando los siguientes iones m/z: 87 (EMD), 115 (EDD), 95 (MIB) y 112 (GSM). Se obtuvo una buena correlación lineal (0.9992-0.9997) hasta 50 µg/l. Los límites de detección fueron similares a los descritos para GSM y MIB por otros autores con esta técnica<sup>[4-5]</sup>, y la desviación estándar relativa a una concentración de 0.5 µg/l se situó entre el 5 y 10%. La exactitud del método y la ausencia de efecto matriz se comprobaron con un estudio de recuperación. Por último, se analizaron varias muestras de aguas naturales en las que no se detectó ninguno de los analitos.

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**POTENCIAL DE LA ESPECTROMETRÍA DE MASAS CON IONIZACIÓN DE DESCARGA DE BARRERA DIELECTRICA EN MODO NEGATIVO**

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La descarga de barrera dieléctrica (DBD) ha sido utilizada como método de ionización espectrometría de masas con diferentes formatos entre las que se incluyen la cromatografía de líquidos y la cromatografía gases y las sondas de análisis directo [1]. La DBDI ofrece la posibilidad de generar tanto iones negativos como positivos a través de la intervención de forma simultánea de varios mecanismos de ionización como la captura electrónica y la transferencia de protones. Sin embargo, la mayoría de trabajos publicados sobre DBDI están centrados en el modo de ionización positivo. En este trabajo se explora la capacidad del acoplamiento cromatografía de líquidos/espectrometría de masas con fuente DBDI (LC-DBDI-MS) en modo negativo para ionizar compuestos de diferente naturaleza. Para este propósito se seleccionaron sustancias representativas de diferentes familias: 23 pesticidas organoclorados, 8 éteres polibromados, fármacos antiinflamatorios no esteroideos (NSAIDs) y 3 compuestos fenólicos. El acoplamiento de la fuente DBDI se hizo a través de la modificación de una fuente comercial de ionización química a presión atmosférica (APCI Agilent) de manera que el eluyente procedente del LC es nebulizado y evaporado de la misma manera que en APCI. El cromatógrafo de líquidos estaba acoplado a un espectrómetro de masas con analizador de tiempo de vuelo (Agilent 6220 TOF) trabajando en modo de ionización negativo. Las condiciones experimentales de LC-DBDI-TOFMS (fases móviles y parámetros de fragmentación) se optimizaron por separado para cada familia de compuestos ya que cada una requería diferentes parámetros para obtener una señal óptima. Primero se optimizaron los parámetros referentes a la sonda DBDI así como las condiciones experimentales del espectrómetro de masas.

Se recogieron los espectros individuales de cada uno de los compuestos seleccionados. Los resultados mostraron que el empleo de la DBDI en modo negativo permitía la ionización de analitos que no son ionizables por electrospray (ESI) como algunos compuestos organoclorados y polibromados, incluso aquellos que no contienen H en su estructura. Fue muy común la presencia del ión formado por el intercambio de un halógeno (X) por oxígeno  $[M-X+O]^-$  lo que está en consonancia con los resultados publicados por Schütz y col. [2], donde este ión se formaba en compuestos perfluorados debido probablemente a la colisión con iones  $N_2^+$  generados en el plasma. Por otro lado, al igual que el electrospray, la DBDI es capaz de ionizar compuestos mediante deprotonación  $[M-H]^-$  del grupo hidroxilo como ocurrió con los compuestos fenólicos y los NSAIDs. El proceso de ionización está afectado por el gas de descarga (He en este caso), la afinidad protónica, el potencial de ionización y la naturaleza del compuesto, lo que implica la aparición al mismo tiempo de varios mecanismos de ionización en un proceso complejo. En este sentido se pueden proponer diferentes mecanismos de ionización para DBDI en modo negativo, como son la captura electrónica (CE), CE disociativa, transferencia de protón y formación de aductos. Quizás esto justifica parcialmente porque la fuente de ionización DBDI presenta una mayor cobertura de ionización de analitos con distintas propiedades fisicoquímicas en comparación con las otras fuentes de ionización comerciales más comúnmente utilizadas (APCI, ESI).

**Agradecimientos**

Los autores agradecen el apoyo de la Junta de Andalucía" (Project Ref. FQM-2242) y del Ministerio de Economía y Competitividad (CTQ-2012-34297 y CTQ-2015-71321-P).

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# **BIODEGRADATION SCREENING OF ANTIPSYCHOTIC AND ANTIDEPRESSANT DRUGS BY AN IN VITRO ASSAY USING AN ACTIVATED SLUDGE INOCULUM**

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Among emerging pollutants, drugs are probably the main concern of regulatory authorities due to their huge consume. Drugs, their metabolites and their degradation products could reach the environment mainly due to poor removal rates in Waste Water Treatment Plants (WWTP), and by improper disposal of unused medicines. Removal of pharmaceuticals in WWTPs is mostly restricted to biodegradation and to abiotic processes as oxidation and sorption [1]. Therefore, the biodegradability, which is defined as the capacity of a molecule to be degraded by microorganisms forming more simple final products, is an essential parameter to know the risk that involves the dispersion of a compound into the environment.

Antipsychotic drugs are used to manage psychosis principally in schizophrenia and bipolar disorder. Antidepressants are drugs used for the treatment of major depressive disorder and other conditions, including dysthymia, or anxiety, obsessive compulsive and eating disorders. Due the increasing use of these drugs, they could cause unpredictable health and environmental problems if they are not efficiently removed in WWTPs.

The present communication reports a biodegradation screening of 15 antipsychotic and 8 antidepressant drugs. Biodegradation assays were performed in batch mode using a minimal salts medium inoculated with an activated sludge (collected from a Valencian WWTP) as matrix during seven days. In these in vitro simulation tests, the drug concentrations were monitored by means of HPLC methods using a C<sub>18</sub> analytical column and UV detection. Biodegradation, expressed as percentage, BD(%), was calculated for each compound (Fig. 1).

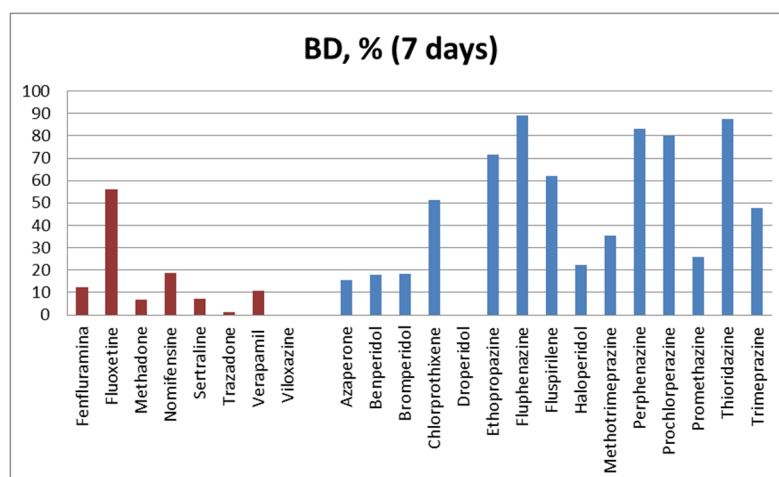


Fig 1.- BD values at 7 days for 15 antipsychotic (in blue) and 8 antidepressant drugs (in red).

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This work has been supported by the Project CTQ2015-70904-R (MINECO/FEDER, UE).

**ALTERNATIVA MEDIOAMBIENTAL Y VALOR AÑADIDO  
DEL COMPOST DE RESIDUOS MUNICIPALES**

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La modernización de la sociedad implica un incremento de la producción de residuos municipales (RM), lo cual es muy importante y fundamental poder realizar una recolección selectiva de los mismos y poder diseñar un sistema de gestión que les permita una alternativa de su uso posterior e incrementar el valor añadido de los mismos. Así, se ha planteado el siguiente trabajo tomando la gestión de los residuos sólidos urbanos con la preparación de “compost de RM” y el objetivo ha sido analizar la composición físico-química del compost preparado. Para ello, se ha elegido una planta de RM en la que se han preparado tres pilas usando como materia prima los RM domésticos que previamente le han sido eliminados todo tipo de inertes. Control y estudio de las concentraciones de elementos minerales que actúan como nutrientes y/o con carácter tóxico para su uso en medio ambiente, del compost durante el proceso de compostaje. Los resultados muestran que el compost de RM obtenido presenta contenidos en elementos minerales (N, P, K, Ca, Mg, Fe, Cu, Zn y Mn) similares a los obtenidos por otros investigadores sobre compost de subproductos de las industrias agroalimentarias <sup>[1]</sup> que confirma su uso para fertilización en agricultura. Así mismo, los niveles de elementos como Ni, Pb, Zn y Cr se encuentran dentro del intervalo según normativa <sup>[2]</sup> clasificando al compost de RM elaborado dentro de la categoría de compost A. Y desde el punto de vista de calidad medioambiental, el compost preparado también cumple con los requisitos exigidos al presentar niveles de polifenoles inferiores a la normativa (<0.8%) <sup>[2]</sup>. Las concentraciones de Na y K fueron tales que en ningún caso la aplicación de este compost de RM puede originar un estrés salino para el cultivo. Por lo tanto, la elaboración de compost de RM sería una alternativa medioambiental para la gestión de los residuos domésticos.

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**VARIABILIDAD DE LAS CARACTERISTICAS FISICO-QUIMICAS  
DE PILA DE COMPOST DE RESIDUOS MUNICIPALES**

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La elaboración de compost tomando como materia prima los residuos domésticos<sup>[1]</sup>, ha sido en la última década una alternativa de gestión muy aceptada para este tipo de residuos que tanta problemática presenta para el medio ambiente. Ahora bien, durante el proceso de compostaje de los mismos nos encontramos con múltiples inconvenientes entre los que cabe destacar la heterogeneidad de la mezcla que se realiza con estos residuos para obtener finalmente un compost de residuos municipales (RM) de calidad. Así, en la realización de este trabajo se planteó el objetivo de analizar la variabilidad que presenta una pila de compost de RM durante su proceso de compostaje. Para ello, se han seleccionado tres pilas diferentes de la misma planta de compostaje y se han tomado muestras en tres posiciones diferentes, izquierda-centro-derecha. Los parámetros a analizar son aquellos que caracterizan físico-químicamente al proceso de compostaje<sup>[2]</sup> e informa de la evolución del mismo. Estas características han sido: pH, CE, C/N, temperatura, humedad, N, P, K, Na, Ca, Mg, Fe, Cu, Zn, Mn, Ni, Cr, Pb. Los resultados mostraron que no había diferencias en los contenidos de la mayoría de los parámetros analizados en función de la posición de toma de muestras en los primeros 87 días del proceso de compostaje. A partir de este momento, de forma general, se observaba una tendencia decreciente del contenido de las características analizadas en la dirección de los extremos. Esto es consecuencia de la dificultad para una óptima homogeneización de la mezcla de las materias primas durante las etapas de volteo y riego. Diferenciándose así tres posiciones a tener en cuenta a la hora de caracterizar la evolución de un proceso de compostaje de RM. Podemos concluir que como protocolo de toma de muestras en compost de RM se debe de incluir la necesidad de obtener una muestra homogénea y representativa de la pila de compost obtenida a partir de la mezcla de tres submuestras recogidas en los extremos y centro de la misma.

<sup>[1]</sup> Ley 22/2011 de 28 de julio, de residuos y suelos contaminados (BOE núm. 181/2011)

<sup>[2]</sup> Antonia Fernández-Hernández, Asunción Roig, Nuria Serramiá, Concepción García-Ortiz Civantos, Miguel A. Sánchez-Monedero. Waste Management 34 (2014) 1139–1147.

## EXPOSICIÓN DE *PROCAMBARUS CLARKII* A PRINCIPIOS ACTIVOS FARMACOLÓGICOS. ACUMULACIÓN EN MUSCULO ABDOMINAL Y HEPATOPÁNCREAS.

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El objetivo del presente trabajo es el estudio de la acumulación de principios activos farmacológicos en muestras de músculo abdominal y hepatopáncreas de la especie animal *Procambarus Clarkii* procedentes de la zona de la Janda (Cádiz).

La especie no autóctona *Procambarus clarkii* (*P. clarkii*) se encuentra ampliamente distribuida en diferentes ecosistemas del SO de la Península Ibérica y es una especie de interés para su empleo como bioindicador de estrés ambiental, dado que las propiedades fisiológicas del mismo permiten la absorción de contaminantes en su organismo.

En el presente trabajo, se han llevado a cabo una serie de ensayos de exposición sobre *P. clarkii* en los que estos organismos fueron expuestos a concentraciones de 100 µg/L y 10 µg/L de una mezcla ibuprofeno:flumequina:ciprofloxacina; a fin de evaluar su acumulación en músculo abdominal y hepatopáncreas. Para ello, se aplicó y optimizó un procedimiento analítico para la determinación de los tres principios activos mediante UPLC-Q-TOF-MS/MS. La separación cromatográfica se llevó a cabo en una columna Acquity BEH C<sub>18</sub> (50x 2,1 mm I.D., de 1,7 µm de tamaño de partícula) termostaticada a 25 °C y una fase móvil constituida por agua (A) y acetonitrilo (0,1% en ácido fórmico) (B) en modo gradiente durante 8 minutos. Se pasó de una composición inicial 90% A al 45% A en 4.5 minutos manteniendo este porcentaje de A durante 3.5 minutos para volver inmediatamente a condiciones iniciales, esperando 2 minutos entre inyecciones. Las muestras se mantuvieron termostaticadas a 5 °C.

Los ensayos de exposición se llevaron a cabo sobre *P. clarkii*, capturados en el entorno de la comarca de La Janda (Cádiz). Para ello, los cangrejos fueron separados en grupos de 35 a 40 ejemplares diferentes en estanques y sometidos a un proceso de aclimatación de 10 días previos al ensayo tras su captura. Para la realización de la exposición, el agua de los estanques se sustituyó cada 48-72 horas, por agua fortificada a los niveles de concentración más arriba indicados en cada uno de los analitos de la mezcla objeto de estudio. Los organismos fueron expuestos a tiempos de 1, 7 y 21 días tras los cuales estos fueron sacrificados y diseccionados para su posterior análisis. Adicionalmente, un grupo de los especímenes de 21 días de exposición fueron sometidos a un proceso de depuración de 7 días tras el cual, los organismos fueron sacrificados y analizados.

Se recogieron muestras del agua a fin de controlar la variación de concentración en fármaco en los estanques. Los cangrejos fueron retirados de los tanques y sacrificados transcurridos los días de exposición. Posteriormente, se diseccionaron obteniendo el músculo abdominal y el hepatopáncreas. Para el análisis de los contenidos en principio activo inalterado, las muestras fueron liofilizadas y extraídas mediante aplicación de energía microondas (5 min, 50 W potencia) utilizando para ello como extractante 2 mL de una mezcla acetonitrilo:agua 50:50 (v/v) a la que se añadió 10 µL de proteinasa-k y 1 µL de ácido fórmico puro, posteriormente se centrifugaron a 7000 rpm durante 10 min, se evaporaron hasta casi sequedad bajo una corriente de N<sub>2</sub> y se reconstituyeron con 1 mL de ácido fórmico al 0.1%(v/v).

**Agradecimientos:** Los autores agradecen al Ministerio de Economía, Innovación y Competitividad la financiación para el desarrollo del proyecto CTM2015-67902-C2-1-P, CTM2016-75908R y FEDER project UNSE10-1E-429.

**EXPOSITION ASSAY OF *LAVANDULA DENTATA* TO SIX DRUGS.  
ACCUMULATION IN LEAF, ROOT AND STEM.**

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Miguel Ángel Bello-López<sup>(1)</sup>, R. Fernández-Torres<sup>(1),(2)</sup>**

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To date, the concerns about accumulation and presence of emerging contaminants in plant matrices has focused mainly on assessing the presence and effects on food crops that may have consequences for human health through the food chain [1]. The purpose of the present work was the evaluation of the accumulation of six drugs on different parts of the selected plant *Lavandula dentata*. This vegetal specie was selected for the assay because its presence is highly extended in Doñana's National Park which is a protected area in the south of Spain in which studies about accumulation and presence of emerging contaminants are being carried out (CTM2015-67902-C-1-P).

In the present work, we describe a procedure based on microwave assisted extraction for the determination of 6 pharmaceuticals: flumequine (FMQ), carbamazepine (CBZ), ciprofloxacin (CPR), enrofloxacin (ENR), diclofenac (DCL), and ibuprofen (IBU) from samples of *Lavandula dentata* and subsequent determination by liquid chromatography-quadrupole time of flight mass spectrometry (LC-QTOF). Analytical recoveries ranged from 60 to 107 % with relative standard deviation (RSD) lower than 15 %. Limit of quantitation (LOQ) values for the 6 pharmaceuticals were in the range 25.8-119 ng g<sup>-1</sup>.

Extraction of 0.5 g vegetal lyophilized sample was achieved with microwave energy at 50 W power for 5 minutes using a mixture acetonitrile: H<sub>2</sub>O (50:50 v/v) as extracting solvent. Subsequently, the extracts were centrifuged for 20 minutes at 6000 rpm, the supernatant taken for further five-fold dilution with an acetonitrile:H<sub>2</sub>O (50:50 v/v) solution. Diluted extracts were microfiltrated through 0,2 µm before LC-ESI-QTOF-MS injection.

Eight plants of 30-40 cm length were used for the exposure assay at three concentration levels: 10 ng mL<sup>-1</sup> (low level of exposition); 700 ng mL<sup>-1</sup> (medium level of exposition) and 10 µg mL<sup>-1</sup> mL (high level of exposition). Two plants were selected as blanks which were watered with tap water. Two plants per level were watered with 10 mL of solution containing all the analytes in the concentration described, every 3 days for one month. The low volume of water used assured wet conditions for the plants and that all of the solution containing the analytes remained in the soil until the next water addition. The plants watered by 10 µg mL<sup>-1</sup> solution, survived only 20 days (sampling was made at 20 days) whereas the watered at 10 ng mL<sup>-1</sup> and 700 ng mL<sup>-1</sup> remained alive for all the assay. Once the assay was finished, the plants were submitted to the procedure described above and finally analyzed (in triplicate per plant) in the UPLC-QTOF instrument. Flumequine and carbamazepine were present in leaf and stem samples nearly in all the exposure levels, being higher concentrations at high level exposure.

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**Acknowledgments:** Authors acknowledge to "Dirección General de Investigación y Gestión del Plan Nacional de I+D+i (Ministerio de Economía, Innovación y Competitividad) Project CTM2015-67902-C2-1-P y FEDER project UNSE10-1E-429.

## DETERMINATION OF MERCURY IN ENVIRONMENTAL SAMPLES OF THE NATURAL PARK OF ESPADÀ

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An old abandoned mining area in the Natural Park of Espadà has been studied with the aim of evaluate a possible contamination in plants, soils and water by mercury as a consequence of this activity. Different zones of sampling near the mine, smelter, a flood without water and a dam nearly the town of Chóvar have been selected in order to determine if the content of mercury is related to or not with the mining activity. Soil samples are taken by samplers of 20 cm, were dried at 35°C during 48 hours and the fraction of 2 mm separated with a sieve were triturated with a ball mill. Samples of plants are cleaned with distilled water in ultrasonic bath during 2 minutes and after that are dried at room temperature during 24 hours for after are separated and crushed with a domestic mill.

A direct mercury analyser based in atomic absorption spectroscopy was used to determine the total content of Hg. This instrument provides results with the minim preparation of sample avoiding possible previous contamination and consequently lower reagent consumption. In the analysis of soils is observed a high contamination of mercury, for that we have decided to determinate the volatile Hg [37-99%] bioavailable [1-10%] through a previous specific treatment and a characterization of pH [5.0-9.6], conductivity [63.1-125.0], humidity [0.13-0.9], organic material [1.1-4.4%] and other parameters. Regarding the analysis of plants, it was found a considerable high content of Hg principally in leaves as compared with stems which could be be consequence of aerial contamination, fact that remark the possible danger of this high concentrations in gas phase for the living beings of the park. Finally, the analysis of water quantifies high content of Hg but lower than legal limit of water for human consumption.

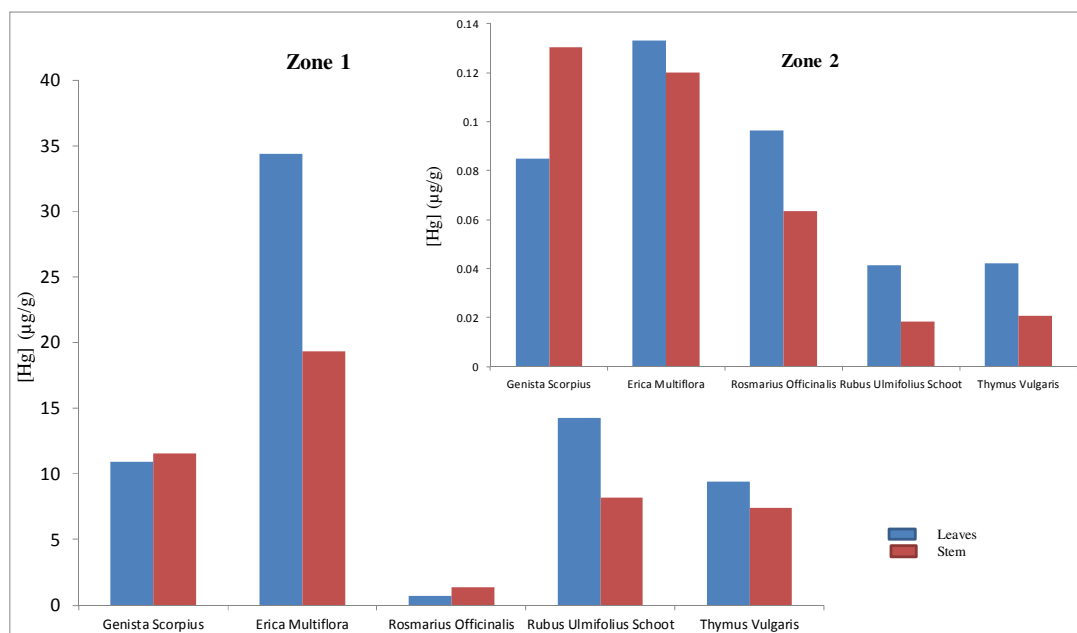


Figure 1.- Comparative of total mercury content in plants in two zones, zone 1 nearly mina Diana and zone 2 in a flood more far of the mining activity.

Acknowledgments: The realization of this project has been possible thanks to the financing by the Generalitat Valenciana Project PROMETEO II 2014/077 and Vicerectorat de polítiques de Formació i Qualitat Educativa Projecte UV-SFPIE\_GER16-417979



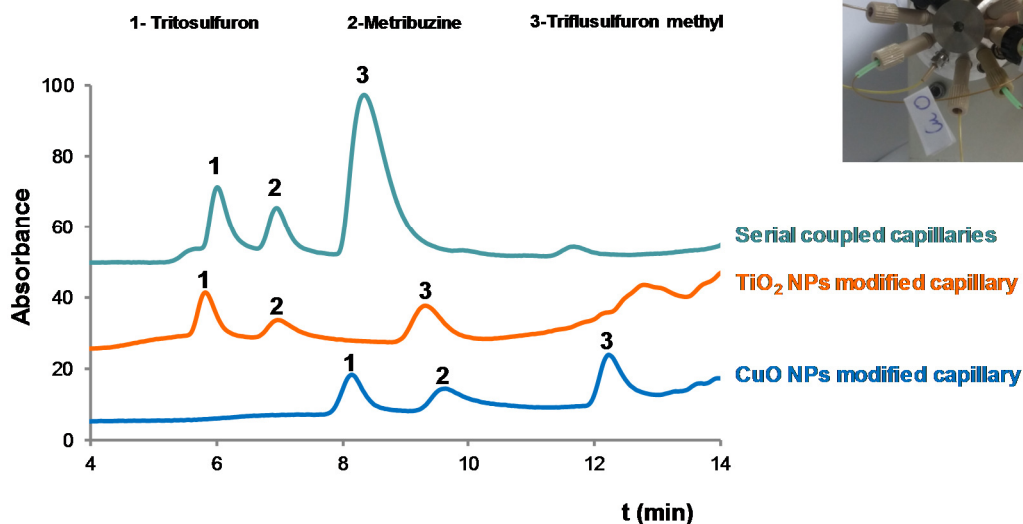
## COMBINED METAL OXIDE NANOPARTICLES MODIFIED COATINGS FOR THE EXTRACTION OF POLAR HERBICIDES BY IN-TUBE SOLID PHASE MICROEXTRACTION

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The employment of hybrid phases for solid-phase (micro)extraction is a very attractive option to improve the extraction efficiency through the combination of different mechanisms of interaction with the target analytes. One of the most successful examples is the functionalization of polymeric coatings with nanoparticles NPs. Because of their high specific area, NPs increase the sites available for interaction with the analytes thus improving the extraction efficiency; under certain conditions, the selectivity can also be enhanced [1].

In this study, capillaries coated with a tetramethyl orthosilicate (TEOS)-trimethoxymethylsilane (MTEOS) polymeric phase have been functionalized with metal oxides NPs and tested for the extraction of highly polar triazine-like pesticides by in-tube (IT-SPME) coupled to nano liquid chromatography. Two types of metal oxide NPs with different selectivity [2] have been used,  $\text{TiO}_2$  NPs and  $\text{CuO}$  NPs. Capillary coatings functionalized with a mixture of both types of NPs have been tested, and the results have been compared with those achieved by the serial coupling of two capillaries, each modified with a single type of NPs, and connected to the analytical column by a 10-port switching valve. The later approach was found more effective, providing increased signals for most of the tested analytes better peak profiles.



Under optimized conditions, eight triazine-like compounds ( $\log K_{\text{octanol/water}}$  partition coefficients ranging from -0.7 to 3.21) were analysed in water. The limits of detection obtained ranged from 0.025 to 1.5  $\mu\text{g/L}$ , the volume of sample being 200  $\mu\text{L}$ . Suitable precision was also achieved, with relative standard deviation, RDSs  $\leq 10\%$  ( $n=3$ ).

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The authors are grateful to Generalitat Valenciana (PROMETEO 2016/109) and to the Spanish Ministerio de Economía y Competitividad (MINECO) and EU-FEDER (project CTQ2014-53916-P) for financial support.



**CALIBRATION CHAMBER FOR THE ESTIMATION OF SAMPLING RATES OF PASSIVE SAMPLERS FOR VOCs SAMPLING FROM AIR****T.D. Ramos<sup>a,b</sup>, R.J. Cassella<sup>b</sup>, M. de la Guardia<sup>a</sup>, A. Pastor<sup>a</sup>, F.A. Esteve-Turrillas<sup>a</sup>**<sup>a</sup> Department of Analytical Chemistry, University of Valencia, Jeroni Muñoz Building, 50<sup>th</sup> Dr. Moliner St., 46100 Burjassot, Spain<sup>b</sup> Departamento de Química Analítica, Universidade Federal Fluminense, Outeiro de São João Batista s/n, Niterói, RJ 24020-141, Brazil  
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Passive sampling technology has been widely used for monitoring pollutants in different environments. The increasing interest on passive samplers in the last years is justified due to their potential as a low-tech and cost-effective monitoring tool. It offers simplicity, easiness and low cost, and it provides the time-weighted average (TWA) concentration, while one of the main drawbacks of the passive sampling technique is the need to calibrate the samplers for individual analytes under known and controlled conditions [1]. Thus, all efforts to approximate laboratory calibration studies to environmental conditions have been made and laboratory calibration experiments are strictly required to allow the correlation between the amount of pollutants present in the device and the respective concentration in the sampled air. A calibration chamber has been designed and employed for the simple and easy determination of uptake sampling rates ( $R_s$ ) of volatile organic compounds (VOCs) from air using passive samplers. A continuous flow of clean air was spiked at a constant VOC concentration by the microinjection of a standard solution by means of a T-type tube. The developed system allowed the complete evaporation of the standard solution and the air concentration of VOCs was easily controlled by the regulation of the clean air flow and the standard solution concentration and flow. Active sampling was employed to monitoring the true concentration of the evaluated compounds in the calibration chamber air, using Tenax-filled desorption tubes and a low flow personal air sampling pump. Versatile, easy and rapid atmospheric monitor (VERAM) devices were employed for the passive sampling of benzene, toluene, ethylbenzene, xylenes,  $\alpha$ -pinene, camphene, myrcene, p-cymene, and limonene from air [2]. The  $R_s$  values obtained for the passive sampling of VOCs, using the proposed calibration chamber, were in the range of 1.3-16.0 m<sup>3</sup> day<sup>-1</sup> in accordance to previous calibration studies performed for VERAM samplers [3]. The developed calibration chamber provides a continuous flow with a constant concentration of the evaluated spiked compounds that allows the simultaneous deployment of several samplers for a rapid establishment of  $R_s$  for a passive sampler and the easy comparison between different devices.

Acknowledgements: The authors gratefully acknowledge financial support of the Ministerio de Economía y Competitividad (Project CTQ 2014 52841) and Generalitat Valenciana (Project PROMETEO-II 2014-077). Also, the authors are grateful to FAPERJ (Fundação Carlos Chagas de Amparo à Pesquisa do Estado do Rio de Janeiro), CNPq (Conselho Nacional de Desenvolvimento Científico e Tecnológico) and CAPES (Coordenação de Aperfeiçoamento de Pessoal de Nível Superior) for the grants and fellowship and for financial support

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# TRACE DETERMINATION OF VOLATILE POLYCYCLIC AROMATIC HYDROCARBONS IN NATURAL WATERS BY MAGNETIC IONIC LIQUID-BASED STIR BAR DISPERSIVE LIQUID MICROEXTRACTION

**Juan L. Benedé<sup>a</sup>, Jared. L. Anderson<sup>b</sup>, Alberto Chisvert<sup>a</sup>**

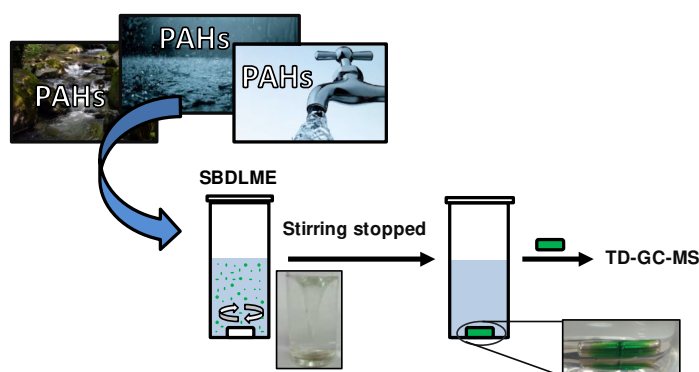
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Sample pretreatment step has a crucial role in the analytical process because of the high amount of handling in which the sample is subjected prior to instrumental determination. In this context, microextraction techniques allow both clean-up (removal of potential interfering compounds from the sample) and preconcentration of the target analytes in only one step, which allows to enhance selectivity and sensitivity.

In this work, a novel hybrid approach called stir bar dispersive liquid microextraction (SBDLME) [1] that combines the advantages of stir bar sorptive extraction (SBSE) and dispersive liquid-liquid microextraction (DLLME) has been employed for the accurate and sensitive determination of ten polycyclic aromatic hydrocarbons (PAHs) in natural water samples. The extraction is carried out using a neodymium stir bar magnetically coated with a magnetic ionic liquid (MIL) as extraction device, in such a way that the MIL is dispersed into the solution at high stirring rate. Once the stirring is ceased, the MIL is magnetically retrieved onto the stir bar, and subsequently subjected to thermal desorption (TD) coupled to a gas chromatography-mass spectrometry (GC-MS) system.

The main parameters involved in TD (i.e., temperature and time), as well as in the extraction step affecting the extraction efficiency (i.e., MIL amount, extraction time and ionic strength) were evaluated. Under the optimized conditions, the method was successfully validated showing good linearity, limits of detection and quantification in the low ng L<sup>-1</sup> level, good intra- and inter-day repeatability (RSD < 13 %) and good enrichment factors (18 – 717). This sensitive analytical method was applied to the determination of trace amounts of PAHs in three natural water samples (river, tap and rainwater) with satisfactory relative recovery values (84 – 115 %), highlighting that the matrices under consideration do not affect the extraction process.



## Acknowledgements

Authors acknowledge the financial support of the Spanish Ministry of Economy and Competitiveness (Project CTQ2015-70301R). JLA acknowledges funding from the Chemical Measurement and Imaging Program at the National Science Foundation (CHE-1413199).

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## CHARACTERIZATION OF ARTIFICIALLY WEATHERED MICROPLASTICS BY ATR-FTIR

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There is currently a steady increase in the global effort spent by the scientific community to better understand the effect of plastics in marine pollution. This is relevant because of the increase on the total amount of plastic litter in marine environments.

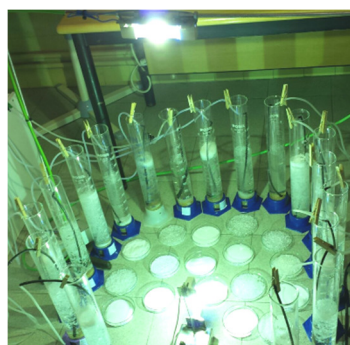
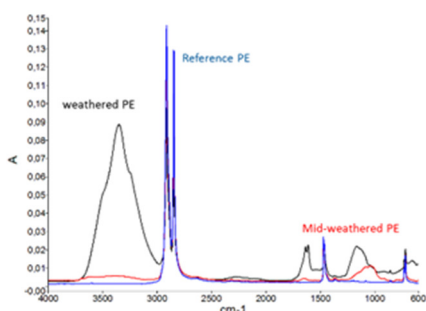
The time required for plastic to degrade in the environment is estimated to be on the order of hundreds to thousands of years, being photo-oxidation by ultraviolet (UV) light their primary degradation pathway [1].

This study focus on the characterization of the changes that an accelerated artificial weathering process in seawater produces in a series of plastics materials using ATR-FTIR. This could be useful to identify real plastics and microplastics found in marine environment, and understand how aging affects the surface and chemical structure of these materials. Knowing how plastic particles weather is important for understanding the ecological impacts of the most common types of marine debris [2].

To this aim, natural microplastics, without additives, in two size presentations (100-500  $\mu\text{m}$  and pellets  $\leq 1$  mm) were artificially weathered. A pilot-scale simulated weathering system, using UV/Vis metal halide lamps, was deployed. Each type of plastic is submerged in sea water in a pyrex glass recipient containing sea-sand. Constant agitation is maintained using an air-aeration pumping system. The containers are irradiated permanently by the lamps during 8 weeks. Accelerated aging of the samples is expected due to the combined effect of mechanical abrasion (sand grinding) and UV/ Vis radiation.

To test the effect of seawater and sand abrasion in the weathering of the plastics, the same plastics were simultaneously exposed to a “dry irradiation”.

The weathering of each plastic was monitored by horizontal ATR-FTIR. New absorption peaks can be seen, that reveal changes in the main structure of the plastics; typically, the broad hydroxyl band from 3100 to 3700  $\text{cm}^{-1}$ . Changes on peaks corresponding to alkenes or C=C bonds (1600-1680  $\text{cm}^{-1}$ ) and carbonyl groups (1690-1810  $\text{cm}^{-1}$ ) can be also observed.



### References:

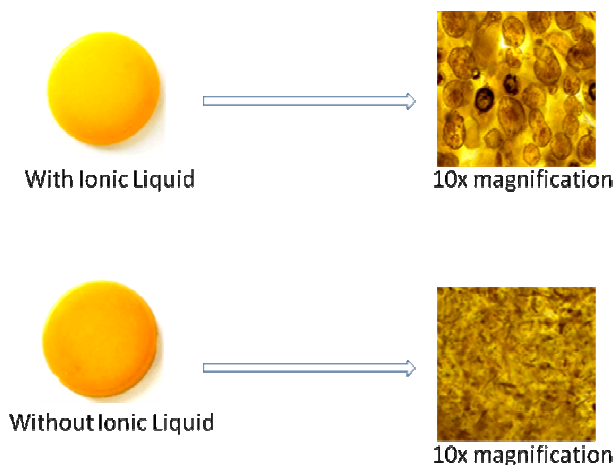
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Aknowledgements: This work has been supported by the Program of Consolidation and Structuring of Units of Competitive Investigation of the University System of Galicia (Xunta de Galicia) partially financed by ERDF in the frame of the operative Program of Galicia 2007–2013 (reference: GRC2013-047). The Ministry of Economy and Competitiveness is also thanked: subproject PCIN-2015-170-C02-01 of the EU-Funded BASEMAN (JPI Oceans) and, project CTM2016-77945-C3-3-R (ARPA-ACUA)

IONOGEL BASED COLORIMETRIC SENSOR TO DETERMINE  $\text{NH}_3$  IN ATMOSPHERES**L.Hakobyan, Y. Moliner Martinez, C.Molins Legua, P. Campins- Falcó**

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Ionogels are a new class of hybrid materials that combine the properties of the polymeric gel and entrapped ionic liquid (IL). The presence of ILs (Fig. 1) in polymeric sensor increment the analyte transfer from the sample into the polymeric matrix and so sensitivity can be enhanced. Moreover, selectivity can be also improved since these sensing platforms show high solvation ability. Their biodegradability and toxicity characteristics are other advantages to improve the greenness of the analytical procedure. In the present work, a new ionogel based on the use of 1-methyl-3-octylimidazolium hexafluorophosphate entrapped on a polydimethylsiloxane-tetraethylortosilicate- $\text{SiO}_2$  nanoparticles composite (PDMS-TEOS- $\text{SiO}_2\text{NPs}$ ) doped with 1,2 naphthoquinone-4- sulfonate(NQS) has been proposed to determinate  $\text{NH}_3$  in atmospheres [1,2]. The sensor detects  $\text{NH}_3$  in the atmosphere by changing the colour from yellow to brown. The intensity of the colour is related to the concentration of  $\text{NH}_3$  in air, and so quantitative analysis was carried out by measuring diffuse reflectance of the sensing membrane after the exposure to ammonia. Different membrane compositions have been tested and analytical responses have been studied as a function of the exposure time. Under the optimum conditions, the presence of ILs in the sensors membrane significantly improved the sensitivity. The LOD with the ionogel was 0.6 ppmv ( $t_{\text{exposure}}=8\text{h}$ ). Meanwhile the LOD with conventional membranes was 2, 4 ppmv ( $t_{\text{exposure}}=8\text{h}$ ). Precision was also evaluated and the relative standard deviation values were up to < 7%. Therefore, the proposed ionogels are a potential alternative to determine ammonia in practical applications.



**Fig.1** Sensors microscopic image with and without IL

The authors are grateful to Generalitat Valenciana (PROMETEO 2016/109) and to the Spanish Ministerio de Economía y Competitividad (MINECO) and EU-FEDER (project CTQ2014-53916-P) and UV valoritza I transfereix program for financial support.

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## EVALUATION OF CAR EXHAUST

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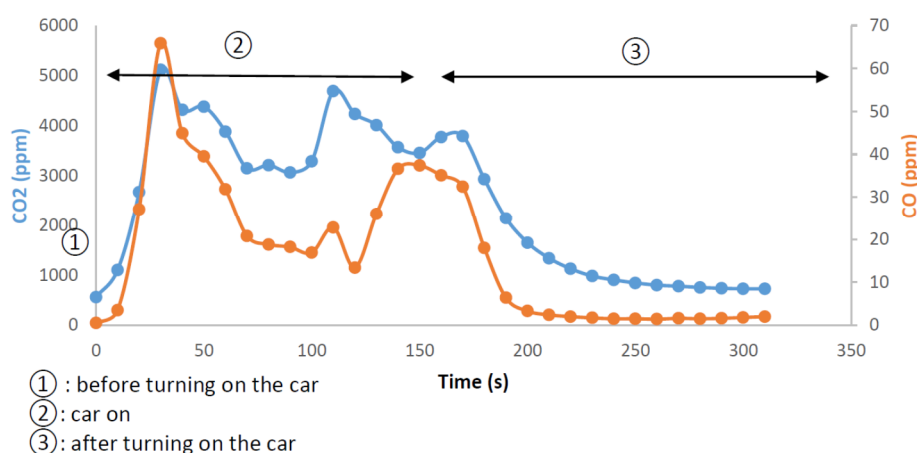
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The composition of the exhaust from diesel engines as NO<sub>x</sub>, VOC, CO, CO<sub>2</sub> and Particulate matter (PM) has been evaluated giving attention to the influence of car characteristics on their parameters. Eleven diesel passenger cars with age from 5 to 19 year, power from 75 to 143 horse power and the number of kilometres from 19439 to 279933 km were evaluated by several monitoring devices.

The measures in this study were taken in different places where each car was parked and all the tests were at 30 ± 2 cm from the exhaust and 20 ± 2 cm from airway which make this method more exact and correct than the other registered ones. The highest level of NO<sub>x</sub> emissions was 7.26 mg/m<sup>3</sup>. CO<sub>2</sub> emissions rates changed within 494 and 5120 ppm. In the case of CO, it has been found that there was a significant divergence, ranged between 0.08 and 152 ppm. For the VOC emissions, the values varied between 0.13 and 62.77 mg/m<sup>3</sup>. Among all vehicle emissions, the values of PM<sub>0.5</sub> present the highest variability compared to PM<sub>2.5</sub> and PM<sub>5</sub> because of their lower mass and smaller size. Additionally, the measurement of PM concentration showed that the values of this parameter varied between 0.07 and 105.88 mg/m<sup>3</sup>. The comparison between emissions of different vehicles has shown that the age, power and the number of kilometres are the most measurable factors which affect the exhaust emissions.

The measurement of human breath before starting up and after turning off the car were used to evaluate the impact of vehicle emissions on the human health. In this step it have been seen a large difference in the values of different parameters between the two measurement moments which reaches a gap of more than 3000 ppm for CO<sub>2</sub>, 16 ppm for the CO, more than 15 mg/m<sup>3</sup> for the PM concentration, more than 2 mg/m<sup>3</sup> for NO<sub>x</sub> and more than 6 ppm for the VOC.

Figure: Variation of CO and CO<sub>2</sub> concentration in a 10 years old car and 112 horse power.



The authors acknowledge the financial support of the Generalitat Valenciana Project PROMETEO II 2014/077, and the Tunisian Private University (ULT)

## EVALUATION OF AIR QUALITY INSIDE A PHYTOSANITARY PLANT

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The air quality inside a working phytosanitary plant was evaluated from the determination of carbon monoxide (CO), carbon dioxide (CO<sub>2</sub>), particulate matter (PM) sizes 0.5, 2.5 and 5 µm, volatile organic compounds (VOCs), nitrogen oxides (NO<sub>x</sub>) and sulfur dioxide (SO<sub>2</sub>) concentration. Measurements were made by using gas sensors in five hot points of a factory in the area of VALENCIA (SPAIN). Solvent and raw material stock (Stk), old (OPr) and modern (MPr) production areas, solid form research and development laboratory (SR&D) inside the plant and liquid form research and development (LR&D) laboratory were controlled.

Maximum concentration levels of CO<sub>2</sub>, PM, VOC and NO<sub>x</sub> for the considerate analytes were 991 ppm of CO<sub>2</sub> and around 58 million of the smallest particles, 22 millions of PM<sub>2.5</sub>, and 8 million of PM<sub>5</sub> in SR&D; 10.05 mg/m<sup>3</sup> of VOC in the OPr and 0.173 mg/m<sup>3</sup> of NO<sub>x</sub> in the SR&D. However, SO<sub>2</sub> and CO was not detected in any area.

Additionally the breath of the experimenter was used as a test of the passive exposure of the people working in the area. The relationship between the present of studied parameter in breath, before/after making measurements inside the factory were 503/603 (CO<sub>2</sub> ppm), 25 million / 21 million (PM<sub>0.5</sub>), 19 million/12 million (PM<sub>2.5</sub>), 38 thousand/50 thousand (PM<sub>5</sub>), 1.32/1.8 (mg/m<sup>3</sup> VOC) and 0.1/0.15 (mg/m<sup>3</sup> NO<sub>x</sub>).

*Table: Summary of gas concentrations detected in the phytosanitary plant factory*

Area		1	2	3	4	5
Analyte						
CO <sub>2</sub> (ppm)	MAX	534	566	657	911	828
	AVG	483	484	553	870	728
	MIN	454	461	478	804	519
VOC (mg/m <sup>3</sup> )	MAX	7.74	10.05	8.36	1.54	5.89
	AVG	5.05	6.99	1.73	1.46	5.16
	MIN	1.03	5.06	1.08	1.06	1.42
NO <sub>2</sub> (mg/m <sup>3</sup> )	MAX	0.101	0.119	0.127	0.173	0.013
	AVG	0.081	0.090	0.092	0.144	0.013
	MIN	0.046	0.066	0.030	0.116	0

The authors acknowledge the financial support of the Generalitat Valenciana Project PROMETEO II 2014/077, and The Tunisian private university (ULT).

**FLUOXETINE AS REFERENCE COMPOUND FOR INOCULUM CONTROL IN  
BIODEGRADATION STUDIES**

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Drugs, their metabolites and their degradation products can reach the environment mainly due to poor removal rates in Waste Water Treatment Plants (WWTPs), and by improper disposal of unused medicines [1]. The biodegradability is an essential parameter to know the risk that involves the dispersion of a compound into the environment.

In a given WWTP, the microbial community of an activated sludge changes with time and, therefore, its potential activity against incoming contaminants. Consequently, published biodegradation (BD) results obtained with a given inoculum, used in a given moment, can just offer the punctual potential capability of the activated sludge; that cannot be extrapolated to different moments. The use of a substance with a well-known BD, in parallel, to the test compound has been recommended in order to verify the capacity of the activated sludge used as inoculum in the biodegradability assays.

In this communication, the possibility of using fluoxetine as a “fast inoculum control” is studied. Fluoxetine was selected because in previous experiments it exhibited relatively high biodegradation (> 60%) in 7 days and relatively low degradation (< 20%) in abiotic conditions. Assays were performed in batch mode using a minimal salts medium inoculated with an activated sludge (collected from a Valencian WWTP) as matrix. Several experiments involving three inoculums (collected in different moments from the WWTP), different storage periods and three different operators were designed. In these *in vitro* simulation tests, the fluoxetine concentrations were monitored by means of HPLC methods using a C<sub>18</sub> analytical column and UV detection. Biodegradation, expressed as percentage, was calculated. A classification of the inoculum capacity was derived.

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This work has been supported by the Project CTQ2015-70904-R (MINECO/FEDER, UE).



## ESPECIACIÓN DE ARSÉNICO CON NANOPARTÍCULAS DE FERRITA Y SU DETERMINACIÓN POR ESPECTROMETRÍA ATÓMICA CON CALENTAMIENTO ELECTROTÉRMICO

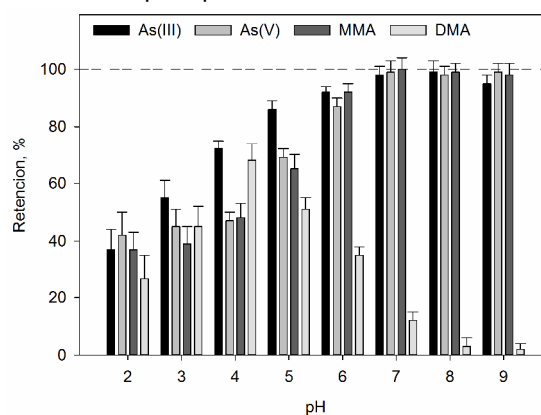
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La World Health Organization (WHO) y la U.S. Environmental Protection Agency (EPA) han establecido un contenido máximo de arsénico de  $10 \mu\text{g L}^{-1}$  en agua de beber [1]. As(V) es la forma predominante en ambientes aeróbicos mientras que As(III) predomina en ambientes ligeramente anaeróbicos y reductores. Otras formas de arsénico orgánico como ácido monometilarsónico (MMA), ácido dimetilarsínico (DMA) y arsenobetaina son más abundantes en muestras agrícolas, marinas y biológicas. Su presencia al nivel de ultratrazas hace casi imprescindible el empleo de alguna técnica de microextracción [2, 3].

La adsorción de estas formas de arsénico en nanomateriales finamente dispersos puede ser una alternativa eficaz al empleo de otras técnicas de microextracción líquida y/o sólida. Es reconocido que los nanomateriales magnéticos conteniendo hierro (FNPs) poseen una gran capacidad para retener arsénico [4] con la ventaja adicional de que tras la adsorción se puede separar fácilmente el material de la disolución aprovechando sus propiedades magnéticas [5]. Existen distintos procedimientos de obtención de FNPs [6]. Uno de los más utilizados por su sencillez es el de coprecipitación. En este procedimiento se mezclan disoluciones acuosas de metales de transición trivalentes y divalentes en relación molar 2:1, respectivamente. La formación de las FNPs se produce al incrementar el pH con disoluciones de hidróxido sódico o amoníaco en agitación vigorosa.

En este trabajo se presenta un procedimiento rápido para la determinación de arsénico total (As(III), As(V) y MMA) y su especiación utilizando un adsorbente de nanopartículas de ferrita, sintetizadas justo antes de su empleo, sin ninguna modificación. Se propone un procedimiento de síntesis de las FNPs basado en la técnica de co-precipitación utilizando relaciones Fe(III):Fe(II) de 1:2 a temperatura ambiente y sin eliminar el oxígeno disuelto. En las condiciones recomendadas DMA y AB no se retienen en las FNPs como se puede apreciar en la figura. La selección de tres condiciones experimentales distintas permite la adsorción diferencial de estas especies sobre la FNPs. La introducción de microsuspensiones del material en el atomizador ha permitido la cuantificación de As(III), As(V) y MA en agua potable entre 0,05 y  $2 \mu\text{g/L}$ . Se ha optimizado el procedimiento para la especiación de estas formas en muestras de agua potable e infusiones de té de origen distinto, manzanilla, tila y poleo. El procedimiento propuesto se ha validado con la determinación de arsénico en diversos materiales de referencia (aguas de distinta naturaleza y materiales de origen vegetal y biológico).



*Este trabajo se ha realizado gracias al apoyo financiero recibido de la Comunidad Autónoma de la Región de Murcia (Fundación Séneca, 19888/GERM/15), MINECO (CTQ2015-68049-R) y Comisión Europea (FEDER).*

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**UNTARGETED METABOLOMICS ANALYSIS REVEALS KEY PATHWAYS RESPONSIBLE FOR THE ANTITUMORAL PROPERTIES OF SELENIUM NANOPARTICLES****E. Cepriá, H. Estévez, E. García-Calvo, J.L. Luque-García**Department of Analytical Chemistry, Faculty of Chemistry, Complutense University of Madrid,  
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Cancer cases worldwide are predicted to increase by 70% over the next two decades. The latest world cancer statistics report that the number of new cancer cases will increase to more than 15 million in 2020.<sup>1</sup> In view of these alarming data, sensitive, rapid and robust analytical methods for diagnosis/prognosis and effective treatments are in urgent need.

The benefits of selenium as a possible chemopreventive or chemotherapeutic agent have been extensively studied because both, inorganic and organic selenospecies, adversely affect the progression of cancer. It is important to consider that the dose and the chemical form of selenium have a significant influence on these effects.<sup>2</sup> Specifically, Se nanoparticles (SeNPs) have shown unique properties as potential antitumoral agent as compared to other selenospecies.<sup>3</sup> However, further investigations are needed to clarify the interaction mechanisms of these SeNPs with cancer cells.

Metabolomics refers to the identification and quantification of the small molecule metabolic products (the metabolome) of a biological system (cell, tissue, organ, biological fluid, or organism) at a specific point in time. Because changes of metabolites and their concentrations are a direct reflection of cellular activity, the use of an untargeted metabolomic strategy, which is based on comparing patterns of metabolites among different samples using chemometric tools, can help to identify metabolic pathways altered under certain conditions, as well as to elucidate their complexity.<sup>4</sup>

In order to identify changes at the metabolome level of cancer cells exposed to SeNPs, we synthesized and characterized chitosan-stabilized SeNPs, that were subsequently added to hepatocarcinoma cells (HepG2) for 72h. After this time, cells were lysed and the metabolites extracted and derivatized prior determination by gas chromatography coupled to a high-resolution time-of-flight mass spectrometer (GC-TOF-MS). Statistical analysis reflected the perturbations in the metabolism (amino acids, organic acids, sugars) of HepG2 cells exposed to SeNPs.

The present study demonstrates that metabolomics can be employed as a tool to get a deeper insight into the potential of SeNPs as antitumoral agent, and has allowed us to identify biochemical pathways involved in the interaction of these NPs with cancer cells.

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## DISOLVENTES BIOSUPRAMOLECULARES.CARACTERIZACIÓN Y POTENCIAL EN APLICACIONES ANALÍTICAS

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En este trabajo se investiga por primera vez el uso de biotensioactivos para la síntesis de disolventes biosupramoleculares (bioSUPRAS). Los SUPRAS son líquidos nanoestructurados que se generan a partir de disoluciones acuosas o hidro-orgánicas de sustancias anfífilas, mediante procesos espontáneos de autoensamblaje y coacervación. Para su síntesis se parte de una disolución de moléculas anfífilas, en la que al superarse una concentración crítica se forman agregados tridimensionales: micelas acuosas, inversas o vesículas. A continuación, por efecto de un estímulo externo (un cambio de temperatura o pH, adición de una sal o un disolvente) los agregados se autoensamblan en una nueva fase con una nano- o microestructura diferente (esponja, lamelar, hexagonal inversa, etc.) que es insoluble con la disolución en equilibrio. Esta nueva fase, SUPRAS, se ha explotado con éxito en extracciones analíticas ya que presenta regiones de diferente polaridad para la extracción de una amplia variedad de analitos. También se comportan como materiales de acceso restringido, lo cual permite realizar la extracción y exclusión simultánea de interferentes comunes en la muestra como, por ejemplo, macromoléculas (proteínas, polisacáridos, etc.).

En este trabajo se investiga por primera vez la síntesis y caracterización de bioSUPRAS, formados por compuestos anfífilos procedentes de una fuente natural (biotensioactivos). Los biotensioactivos más comunes son de origen microbiano, ya que su recuperación y purificación es la más simple y rentable. Esta alternativa a los tensioactivos sintéticos está ganando fuerza en las últimas décadas debido a que presentan numerosas ventajas: mayor biodegradabilidad, baja o nula toxicidad, concentraciones micelares críticas menores, excelente actividad superficial y efectividad bajo condiciones extremas de temperatura y pH. Los ramnolípidos son los biotensioactivos más comunes y disponibles comercialmente. Su estructura se caracteriza por tener una o dos moléculas de ramnosa unidas a uno o dos  $\beta$ -hidroxiácidos, cuya longitud de cadena es variable y son producidos por a partir de *Pseudomonas aureoginosa*. En bibliografía se ha descrito su autoensamblaje en disoluciones acuosas en forma de vesículas, micelas y estructuras laminares. Sin embargo, no existen estudios sobre coacervación o separación de fases hasta la fecha.

En este estudio se diseñan bioSUPRAS de ramnolípidos en presencia de sal como agente coacervante (cloruro de sodio y sulfato de sodio). Se describen los diagramas de fases para definir la región de formación del bioSUPRAS, los volúmenes obtenidos, la composición y la microestructura del mismo. Los bioSUPRAS de ramnolípidos proporcionan fases con un alto contenido en agua (80–50%) y un elevado número de grupos polares (hidroxilo y carboxilo) lo cual les confiere un alto potencial para la extracción de compuestos polares. Además, su carácter “verde” y biocompatible los convierte en candidatos excelentes como alternativa a los disolventes convencionales.

Agradecimientos: Los autores agradecen al MINECO (CTQ2014-53539-R) y a los Fondos FEDER la financiación recibida. E. R.G. agradece al MECD la beca FPU15/03704. A. B.G. agradece a MINECO su contrato Ramón y Cajal (RYC-2015-18482).

**BIOIMAGING OF APP AND CFH PROTEINS IN OCULAR SECTIONS BY FLUORESCENCE AND LA-ICP-MS USING GOLD NANOCLOUDS AS LABELS**

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Age-related macular degeneration (AMD) is a neurodegenerative ocular disease of the aging eye and the leading cause of irreversible blindness among people 65 years or older. There are not efficient therapeutic treatments to stop this condition up to now, even in the early stages. The main hallmark of early AMD is the build up of extracellular deposits, i.e., drusen, in the macular region of the retina, due to a degeneration of the retinal pigment epithelium (RPE). Modulation of the formation of these deposits would help to slow down or stop the progression of AMD. Within this context, our final goal is to elucidate whether the antioxidant system Zinc-Metallothionein (Zn-MT) can be used as a therapeutic target for the treatment of AMD. Based on current knowledge, an impaired Zn-MT redox system occurs during the onset and progression of AMD, which induces cell damage in the RPE through a mechanistic set of events, including intracellular release of free zinc and copper, cytotoxicity, induction of calcium release and uncontrolled protein aggregation, such as amyloid precursor protein (APP) and complement factor H (CFH), in the extracellular space, triggering inflammation, cell death and subsequent drusen formation. Therefore, preventing zinc release through MT regulation could stop drusen formation and progression of RPE cell damage.

Highly sensitive and multiplexed analytical tools are required to assess the synergistic relationship between the impairment of Zn-MT redox system and drusen formation in the RPE in ocular tissues sections. These methodologies must permit the simultaneous localization (bioimaging) of metals and proteins, specifically those involved in the degenerative processes of drusen formation. In this communication, the distribution of APP and CFH will be studied in different ocular regions (RPE, retina, sclera, cornea, etc). Aiming to achieve a high sensitivity and to obtain quantitative results, bioimaging using bioconjugated gold nanoclusters as specific labels has been investigated using both fluorescence and laser ablation (LA) coupled to ICP-MS as detection techniques.

Acknowledgements: Project ref. CTQ2016-79015-R (AIE/FEDER, UE).

## GREEN BIOSYNTHESIS OF TELLURIUM NANOPARTICLES USING TEA INFUSIONS AS A NATURAL SOURCE OF POLYPHENOLS

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In the last few years, techniques for obtaining nanoparticles using naturally occurring reagents have been considered very attractive for nanotechnology. Among all these reagents, plant based materials seems to be more suitable for large-scale biosynthesis of metal nanoparticles (NPs), probably being polyphenolic compounds the active synthesis agents. Thus, polyphenol biomolecules present in plant extracts can be used to reduce metal ions to NPs in a single-step synthesis process [1]. Among natural sources of polyphenols, flavonoids and phenolic acids are of great abundance in tea from the young buds and dried leaves of *Camellia sinensis*, which is one of the most popular and consumed beverages in the world.

Compared to other chemical and physical methods, green synthesis of metal NPs are simple, eco-friendly, nontoxic, economic, relatively reproducible and often results in more stable materials. In addition, these benign biological methods are especially attractive if they are intended for non-invasive applications in medicine [2].

Tellurium is a non-essential and hazardous metalloid with biological and toxicological effects little understood. Tellurium based nanomaterials have attracted increased attention in the nanotechnology industry for their particular characteristics and potential applications in piezoelectricity, photo and thermoconductivity, and non-linear optical responses. Tellurium nanoparticles (TeNPs) have also revealed interesting antimicrobial potential against different pathogenic strains. These open promising applications as coating agents in medical devices, in the drug delivery and the protection of biologically active materials (enzymes or proteins) [3].

In this work, TeNPs have been synthesized using green methods based on tea infusions, including green, decaffeinated green, black and decaffeinated black teas. Firstly, the biosynthesis was performed employing standard solutions of some polyphenols detected on tea infusions, such as quercetin, gallic acid, *p*-coumaric and *trans*-ferulic acids.

Several factors including reaction time, amount and volume of reagents, and stabilizing agents were evaluated at room temperature. Finally, 20 mL of 6 g·L<sup>-1</sup> polyphenol standard solution were mixed with 25 mL of 2% hydroxyethyl cellulose (HEC) and 20 mL of 2.5 g·L<sup>-1</sup> K<sub>2</sub>TeO<sub>3</sub>. The mixture was magnetically stirred for 30 min and the mixed solution diluted to 100 mL with water (final concentrations were 1.2 g·L<sup>-1</sup> polyphenol, 0.5 g·L<sup>-1</sup> tellurite salt and 0.5% HEC (w/v)). Tellurium NPs were only observed by transmission electron microscopy (TEM) when gallic or *trans*-ferulic acid standard solution was employed, with estimated nanoparticle diameters between 40 and 200 nm. Characterization of TeNPs was also done by UV-visible spectrophotometry, Fourier transform infrared spectroscopy (FTIR), attenuated total reflection (ATR) infrared spectroscopy and powder X-ray diffraction (XRD). IR and XRD analyses confirmed the presence of elemental TeNPs in the synthesis made with *trans*-ferulic acid, and the presence of both elemental Te and TeO<sub>2</sub> nanoparticles in those made with gallic acid.

Regarding TeNPs synthesis using tea infusions, similar reaction procedures were employed. Tea amount, infusion volume, reaction time and stabilizing agents were also optimized. Under optimized conditions, analyses by TEM showed the presence of TeNPs when black, decaffeinated black and green teas were employed. Estimated particle diameters were around 80 nm, between 100 and 120 nm, and in the range 2-170 nm, respectively. Decaffeinated green tea did not produce TeNPs and in general, it could be concluded that the presence of caffeine has a negative effect on the amount and size of NPs. Further studies will conduct to assess this effect and to characterize the tellurium nanoparticles by IR and XRD techniques.

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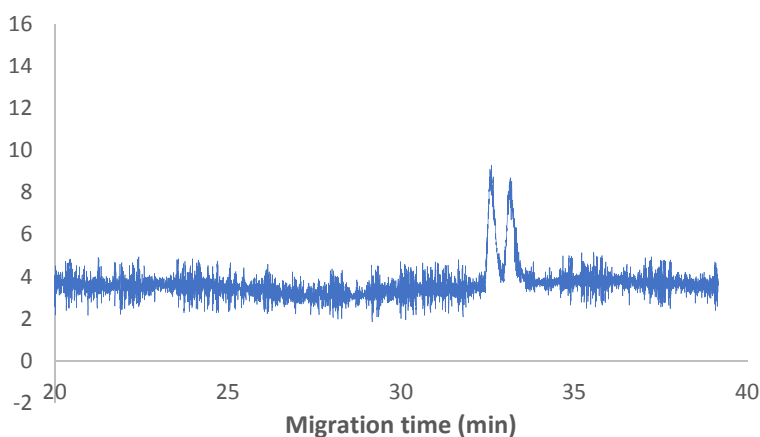
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**USE OF POLY(STYRENE-CO-DIVINYLBENZENE) WITH ATTACHED MAGNETIC NANOPARTICLES AS A SORBENT MATERIAL FOR EXTRACTION OF PROPRANOLOL FROM URINE SAMPLES PRIOR TO THEIR ENANTIOSELECTIVE DETERMINATION BY CAPILLARY ELECTROPHORESIS**

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A simple method was developed for the extraction of R- and S- enantiomers of propranolol, a well-known drug used for its antihypertensive effect, from free-drugs urine samples. The enantioselective determination is carried out by capillary electrophoresis using 2-hydroxypropyl- $\beta$ -cyclodextrin as a chiral selector, formerly reported in literature [1]. A new sorbent material was prepared by co-polymerisation of styrene and divinylbenzene in a 50:50 proportion [2] with the attachment of magnetic nanoparticles to enhance its preconcentration capacity and this was proven to be more effective than commonplace cartridges for classical solid phase extraction. Once synthesised, 0.5 g of the new material were weighed and placed in a home-made cartridge for an easier extraction procedure. This was carried out by following these steps: conditioning with 2 mL of methanol and 6 mL of water, sample loading with an initial volume of 50 mL and elution with 2 mL of methanol prior to partial evaporation under nitrogen stream down to 0.5 mL, being this the final volume. Buffer composition consisted of an aqueous mixture of 80 mM ammonium acetate and 8 mM (2-hydroxypropyl)- $\beta$ -cyclodextrin at pH 3.5, and the use of the cyclodextrin was crucial to separate and differentiate between enantiomers due to their interaction with the buffer. R- and S-propranolol standards were prepared in HCl 0.1 M and a calibration curve was built with concentrations ranging from 50 to 4000 ppb for each of them. Once calibration curves were prepared for each enantiomer, several free-drug urine samples were spiked with both propranolol enantiomers at certain concentration levels within the range assayed (100-400 ppb) to assess the suitability of the extraction method in terms of recovery. For this purpose, an additional cleaning step had to be introduced before elution with the aim of minimising interferences derived from the injection of the urine samples, and 6 mL of water were employed after sample loading to carry on as stated. Recovery values were beyond 85% for every concentration level. The separation is shown in Figure 1.



*Fig. 1. Electropherogram of a free-drug urine sample spiked with S- and R-propranolol at 100 ppb each enantiomer under extraction and electrophoretic conditions above stated.*

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# ASYMMETRIC FLOW FIELD FLOW FRACTIONATION HYPHENATED TO ICP-MS FOR GOLD NANOPARTICLES AND DISSOLVED GOLD SPECIES DETERMINATION IN CELL CULTURE MEDIUM

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The use of nanoparticles (NPs) in different fields has rapidly grown in the last decades. Among them, gold nanoparticles (AuNPs) are widely used for biomedical applications. However, their impacts on the human health and the environment are not fully known. The study of the AuNPs toxicity addresses these concerns, but special attention should be paid to the AuNPs interaction with the matrix components and to the possibility of gold ion ( $\text{Au}^{3+}$ ) release. Therefore, the characterization of NPs in complex matrices is necessary. For this purpose, the coupling of hydrodynamic separation techniques with specific detectors begin to play a decisive role [1]. Asymmetric flow field flow fractionation (AF4) is a recently proposed separation technique with a high potential in the field of NPs analysis. The separation takes place at low-to-medium pressure in an open channel without a stationary phase, so interactions between the NPs and stationary phase or mechanical stress are avoided, which in turn minimizes the generation of artefacts. The combination of AF4 and inductively coupled plasma mass spectrometry (ICP-MS) is an especially promising and interesting option, because of its particle size related power, its versatility, and its elemental specificity. However, its use for the characterization or detection of NPs in complex samples is still scarce.

Therefore, in the present work an analytical strategy based on AF4-ICP-MS has been developed to study AuNPs transformation in cell culture medium used in toxicity tests. Special attention was paid to the optimization of the separation conditions in the AF4. The dimensions of the channel and the characteristics of the membrane play an important role. Based on previous studies a 350  $\mu\text{m}$  thick spacer and a regenerated cellulose membrane (MWCO of 10 kDa) were selected. Moreover, the composition of the carrier is decisive for the NPs stability and the interactions with the membrane. The presence of surfactants (0, 0.01 and 0.05 % sodium dodecyl sulphate (SDS)), phosphate buffer (without and with phosphate buffer 1 mM at pH 7.9), organic solvents (0, 2 and 4 % methanol) and different pH values (6 and 8) were studied. The optimum carrier composition was 0.01 % SDS at pH=6. The cross flow and the gradient time were also studied.

Under the final optimized separation conditions, dissolved  $\text{Au}^{3+}$  and AuNPs of 10 and 30 nm can be separated by AF4-ICP-MS, with a retention times of 8 min, 12.5 min and 18 min, respectively. An increase in the hydrodynamic volume of AuNPs and dissolved  $\text{Au}^{3+}$  was observed in the presence of the cell culture medium, suggesting that AuNPs and  $\text{Au}^{3+}$  could be coated by species such as proteins present in the culture medium. Oxidation of the AuNPs was also observed. The method will be used for the analysis of cells and supernatants obtained from toxicity test with cell culture under different experimental conditions.

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## Acknowledgments:

Project MINECO CTQ-2016-78793-P and pre-doctoral contract MINECO BES-2014-069095.

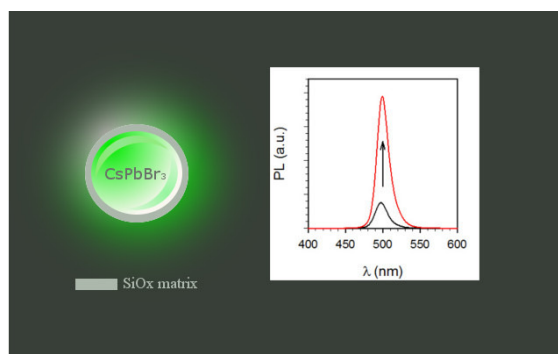
# 4-FOLD PHOTOLUMINESCENCE ENHANCEMENT IN CsPbBr<sub>3</sub> NANOCRYSTALS BY SPECIFIC BINDING AND ENCAPSULATION WITH AMINO-FUNCTIONALIZED SILANES

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Metal halide perovskites (PS) exhibit many appealing features such as high photoluminescence quantum yields, particle size of 5-15 nm, tunable emission properties and giant multiphoton absorption, which may enable fresh approaches for next generation of bioimaging and biosensing applications [1]. Nonetheless, their implementation stumbles with their poor chemical and structural stability in water and limited processability.

In the present work, we describe a facile methodology to prepare cubic green-emitting CsPbBr<sub>3</sub> nanocrystals (NCs) encapsulated in an alcoxysilane shell. TEM microscopy and <sup>1</sup>H-NMR studies enabled to identify the presence of alcoxysilane surrounding the perovskite and elucidate the chemical bonding mechanisms of 3-aminopropyltriethoxysilane on NCs surface. However, the most relevant result of the investigation is the systematic increase in the emission properties observed for the CsPbX<sub>3</sub> NCs treated with alcoxysilane. After the optimization of the methodology, nanocomposite particles exhibit an improvement in photoluminescence close to 400%, compared to uncoated counterparts. This result is of great importance, since it reveals the singular "defect chemistry" of perovskites, where most of the electron recombination phenomena occur on the surface of the nanocrystal, being practically negligible inside the nanoparticle. Therefore, our study reveals the fundamental role of interfacial properties and surface chemistry in modulating the optical properties of CsPbBr<sub>3</sub> nanocrystals. [2]. On the other hand, the silica coating further increases the perovskite's moisture resistance, thus rendering the PS NCs more stable in protic solvents compared to uncoated NCs.



## Acknowledgements

This work was financially supported by FEDER projects BiHolog-CTQ2016-75749-R from MINECO and GVA PROMETEO II 2014/40. V.G-P. thanks Universitat Politècnica de València for her post-doctoral fellowship (Grant-PAID-10-14).

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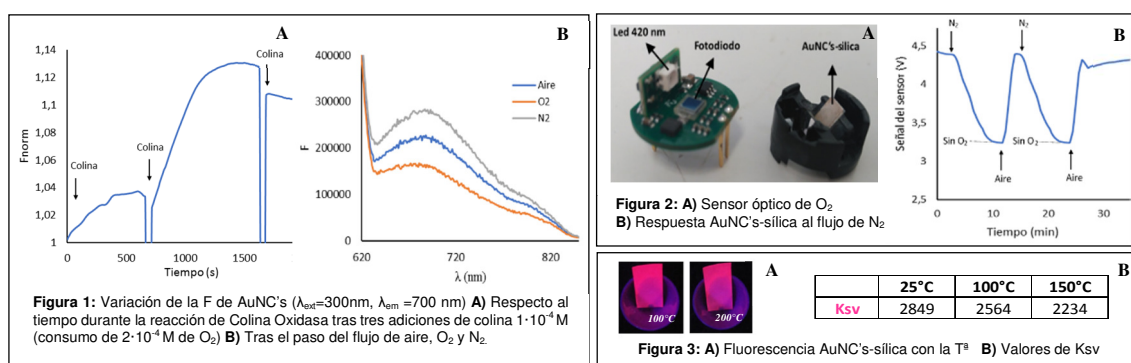
## NANOCLUSTERS DE ORO COMO SONDAS DE OXÍGENO: DESACTIVACIÓN DE LA FLUORESCENCIA Y EFECTO DE LA TEMPERATURA

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Los nanoclusters de oro (AuNC's) son nanoestructuras con tamaños inferiores a 3 nm, formados por agrupaciones de unos cientos de átomos, que presentan un gran atractivo en la investigación debido a sus propiedades físico-químicas únicas, entre las que destacan su fluorescencia en la región VIS-NIR con largos tiempo de vida. En este trabajo se presentan los resultados obtenidos con NC's de oro sintetizados con ácido lipoico como agente estabilizante.

La caracterización fluorescente de estos AuNC's presenta un amplio espectro de excitación ( $\lambda_{ext}=250-450\text{nm}$ ) con un máximo de emisión  $\lambda_{em}=700\text{nm}$  y valores de tiempo de vida altos (120ns), asociados a fenómenos de desactivación fluorescente o quenching. Estas propiedades han llevado al estudio del efecto del oxígeno (desactivador químico) en la fluorescencia de AuNC's, tanto por el consumo de  $O_2$  mediado por una reacción enzimática (Fig.1A) como por paso del flujo Aire- $O_2$ - $N_2$  (Fig.1B), comprobando su efecto desactivador sobre la fluorescencia de estos nanomateriales.



En este punto, los AuNC's se han inmovilizado en un soporte de sílica, para servir como base química de un sensor óptico basado en una plataforma electrónica (Fig.2A) compuesta por una fuente de excitación led de 400 nm y un fotodiodo como detector. La respuesta de la plataforma AuNC's-silica para una misma concentración de oxígeno (Fig.2B), muestra buena sensibilidad, alta reproducibilidad y cortos tiempos de respuesta, que aseguran su posibilidad de aplicación como sondas de oxígeno en la región del NIR. Además, frente a otros fluoróforos, estas nanoestructuras presentan gran fotoestabilidad, menor fotoparpadeo (photoblinking) y buena estabilidad frente a la temperatura debido a su estructura metálica.

Se ha comprobado que la fluorescencia de los AuNC's inmovilizados en el soporte de sílica, permanece hasta  $200^\circ\text{C}$  (Fig.3A), con una Ksv (Ec. Stern Volmer) prácticamente constante entre 25 a y  $150^\circ\text{C}$  (Fig.3B). A partir de  $200^\circ\text{C}$  (entre  $200-700^\circ\text{C}$ ) se observa un cambio en la estructura de los AuNC's. Imágenes TEM confirman la formación de nanopartículas de oro (AuNP's) de dos poblaciones de tamaños, 3 y 11 nm que presentan dos nuevos máximos de fluorescencia con pares  $\lambda_{ext}/\lambda_{em}$ , 290/330nm y 330/415nm, y una banda de absorción en torno a 540nm. La fluorescencia de estas nuevas nanopartículas formadas por efecto de la temperatura (partículas estabilizadas con óxidos de oro en su superficie), está siendo evaluada, observándose en los primeros estudios, respuesta al  $O_2$  para el máximo de emisión de 330nm, que podría atribuirse a la población de NP de 3 nm, con propiedades más próximas a las de los AuNC's originales.

Este trabajo ha sido realizado con cargo al Proyecto CTQ2016-76846-R (MINECO) y a las ayudas a grupos de investigación DGA-FEDER (E74).



## SÍNTESIS “IN SITU” DE NANOPARTÍCULAS DE ORO ACOPLADA A LA DETERMINACIÓN ENZIMÁTICA DE AMINAS BIÓGENAS

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Las nanopartículas de oro son un tipo de nanomaterial metálico muy utilizado en técnicas ópticas de determinación analítica, debido a su capacidad para producir efecto de resonancia de plasmón superficial. Donde más impacto están teniendo este tipo de nanomateriales es en su utilización como sensores, por su capacidad de aportar esquemas indicadores novedosos que, respecto de los colorimétricos y fluorimétricos convencionales, suponen una mejora en sensibilidad, estabilidad y biocompatibilidad, por lo que en un futuro pueden dar lugar a biosensores para la monitorización in vivo de parámetros de interés biomédico y biotecnológico. Los métodos de síntesis de estos nanomateriales son muy variados, siendo uno de los más utilizados, los métodos bottom-up, que incluyen un reductor que transforma el Au (III) en Au<sup>0</sup> y un ligando orgánico para controlar su estabilidad coloidal y evitando su agregación.

En este trabajo se pretende desarrollar una metodología analítica, basada en los métodos bottom-up, mediante la síntesis “in situ” de nanopartículas de oro durante reacciones enzimáticas que involucran procesos redox de la enzima.

El esquema general del proceso es el que se muestra en la figura 1. La enzima en su forma oxidada reacciona con su analito, pasando así a su forma reducida. Normalmente la enzima vuelve a su estado inicial (forma oxidada) utilizando el O<sub>2</sub>, comenzando así de nuevo el ciclo enzimático, sin embargo, en presencia de Au (III), la enzima se regenera reduciendo el Au (III) a Au<sup>0</sup>. Además, al tratarse de una proteína, sirve como agente estabilizante de las partículas de Au<sup>0</sup>, formándose así las nanopartículas de oro. Por otra parte se ha comprobado que la formación de estas nanopartículas también depende del tipo de analito utilizado, por lo que el mecanismo de la reacción no está del todo claro.

Este esquema general ha sido utilizado con éxito en diversas reacciones enzimáticas entre las que se encuentran la reacción de la diamino oxidasa (DAO) y la reacción de la tiramina oxidasa (TAO). En el caso de la TAO se ha conseguido la determinación específica de tiramina (figura 2). Por otro lado con la DAO, a diferencia de lo que sucede con los métodos colorimétricos tradicionales, ha sido posible la determinación diferenciada de putrescina y cadaverina.

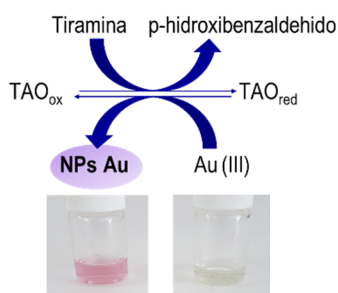


Figura 1: Esquema de síntesis de nanopartículas de oro acoplada a la reacción enzimática de la TAO.

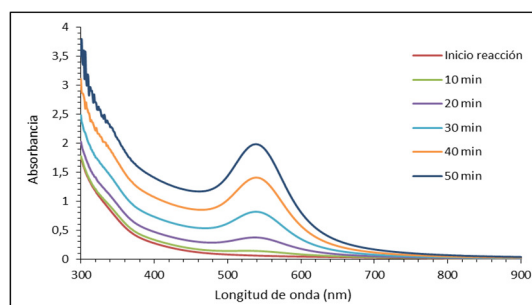


Figura 2: Formación de nanopartículas de oro en función del tiempo.

Hasta la fecha se han conseguido intervalos lineales entre 0,01 mM y 1 mM en todos los casos. En estos momentos se está evaluando la intervención del O<sub>2</sub> en el proceso de formación de las nanopartículas, ya que la cinética de la reacción se ve modificada en condiciones anaerobias.

Este trabajo ha sido realizado con cargo al Proyecto CTQ2016-76846R (MINECO) y las ayudas a grupos de investigación DGA-FEDER. Jesús Navarro agradece a la DGA por la concesión del contrato predoctoral en formación.

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**DEGRADACIÓN DE FÁRMACOS EN AGUA MEDIANTE FOTOCATÁLISIS PLASMÓNICA**

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Hoy en día la calidad del agua es uno de los temas medioambientales más relevantes. Sin embargo, en la última década ha surgido un grupo de contaminantes emergentes en las aguas: los fármacos y los productos de cuidado personal, lo que ha provocado una preocupación generalizada por la falta de conocimiento de sus efectos potenciales en los seres humanos y el medio acuático. Hay que señalar que en países como España y Alemania se han detectado más de 30 fármacos en el agua de consumo<sup>[1]</sup>. Este problema se ha visto acentuado por los residuos procedentes de industrias farmacéuticas y el exceso de medicamentos consumidos por la sociedad moderna, entre los que destacan los antiinflamatorios no esteroideos (NSAID) como el paracetamol, ibuprofeno y ácido acetilsalicílico, utilizados también con fines veterinarios. Por este motivo han adquirido mucha importancia las nuevas tecnologías para el tratamiento de aguas que permitan su reutilización. Entre ellas, la fotocatalisis heterogénea que emplea diferentes catalizadores como  $\text{TiO}_2$  y luz UV (4 % de la radiación solar) para degradar los fármacos hasta  $\text{CO}_2$  y  $\text{H}_2\text{O}$ <sup>[2]</sup>. En los últimos años ha surgido la fotocatalisis plasmónica, que aprovecha la absorción de la radiación visible por parte de nanopartículas de oro o plata soportadas sobre nanomateriales de tipo metal-semiconductor o metal-aislante permitiendo una mejora en la actividad fotocatalítica de los nanomateriales.

En esta comunicación se muestran y comparan los resultados obtenidos en la degradación de dos NSAID, paracetamol e ibuprofeno, usando  $\text{TiO}_2$ -luz UV y una nueva alternativa mucho más rentable económicamente, que usa luz visible y que consiste en el uso de diferentes nanomateriales de oro sintetizados a partir de compuestos organometálicos y soportados sobre  $\text{TiO}_2$ ,  $\text{Al}_2\text{O}_3$  y g- $\text{C}_3\text{N}_4$ .

El seguimiento de las reacciones de degradación fotocatalítica se ha realizado mediante cromatografía de líquidos con detección ultravioleta, observándose cinéticas de degradación de primer orden. Para la detección e identificación de los subproductos generados durante la degradación de los fármacos se ha utilizado un cromatógrafo de líquidos de ultra resolución acoplado a un espectrómetro de masas en tándem cuadrupolo-tiempo de vuelo (UPLC/QTOF-MS). La ionización se realizó con una fuente ESI en modo negativo y positivo.

Se ha conseguido degradar tanto el paracetamol como el ibuprofeno con luz UV y luz blanca (visible) con diferentes nanomateriales. El uso de un detector de masas de alta resolución ha permitido obtener masa exacta de los subproductos detectados y realizar hipótesis sobre los mecanismos de degradación que tienen lugar empleando los diferentes catalizadores.

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## DETERMINACIÓN ESPECTROFOTOMÉTRICA DE CADAVERINA BASADA EN EL USO DE NANOPARTÍCULAS DE ORO ESTABILIZADAS CON RECEPTORES MACROCÍCLICOS.

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En los últimos años, el desarrollo de métodos analíticos basados en la interacción de un determinado analito con nanopartículas funcionalizadas con macrociclos se ha visto incrementado notablemente [1-4]. Los receptores macrocíclicos como los Cucurbit[n]uriles (CB[n]s) o las Ciclodextrinas (CDs) son hospedadores con la capacidad de reconocer compuestos de interés analítico a través de interacciones anfitrión-huésped y, por tanto, pueden ser utilizados en el desarrollo de métodos de detección y determinación rápidos, sensibles y selectivos. Si bien ambas familias de receptores presentan una cavidad hidrófoba accesible por dos entradas, los CB[n]s presentan una estructura simétrica mientras que las CDs presentan una estructura de cono truncado. Además, los portales de los CB[n]s tienen grupos carbonilo que favorecen la estabilización de complejos de inclusión con compuestos cargados positivamente.

En este trabajo se presenta la síntesis y caracterización de nanopartículas de oro estabilizadas con estas dos familias de hospedadores que serán utilizadas para la determinación de cadaverina (1,5-pentanodiamina). El método analítico desarrollado se basa en la capacidad de los CB[n]s y CBs en reconocer diaminas. La interacción entre los receptores y la cadaverina produce un desplazamiento en la longitud de onda del plasmón producto de la agregación de las nanopartículas, acompañado de un cambio en el color de la suspensión de rojo a azul (Figura 1). La determinación de cadaverina se llevó a cabo monitorizando el desplazamiento de la longitud de onda de las AuNPs.

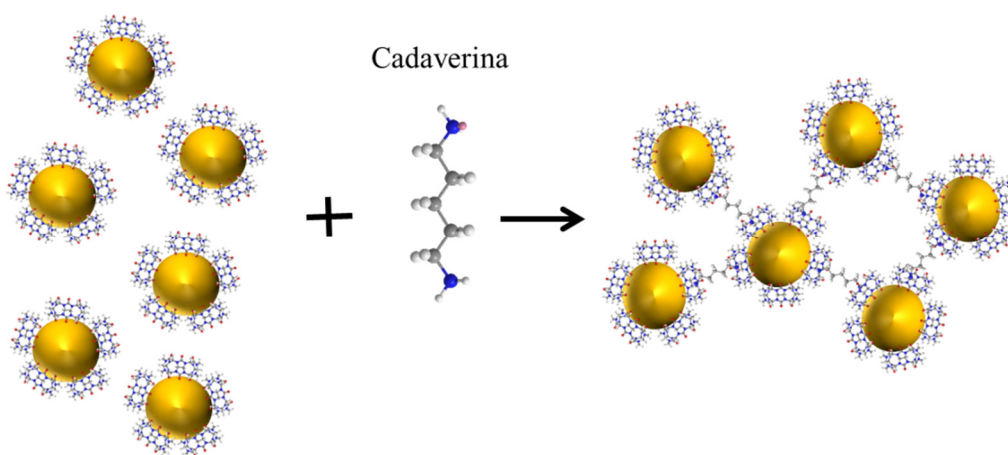


Figura 1

En este trabajo se compara la respuesta del analito con nanopartículas estabilizadas con los homólogos, CB[6], CB[7],  $\alpha$ -CD y  $\beta$ -CD. Se observa que tanto el tamaño como la naturaleza del receptor juegan un papel importante en la interacción de las nanopartículas con la cadaverina.

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**RECYCLING POLYMER RESIDUES TO SYNTHESIZE MAGNETIC NANOCOMPOSITES FOR DISPERSIVE MICRO-SOLID PHASE EXTRACTION**

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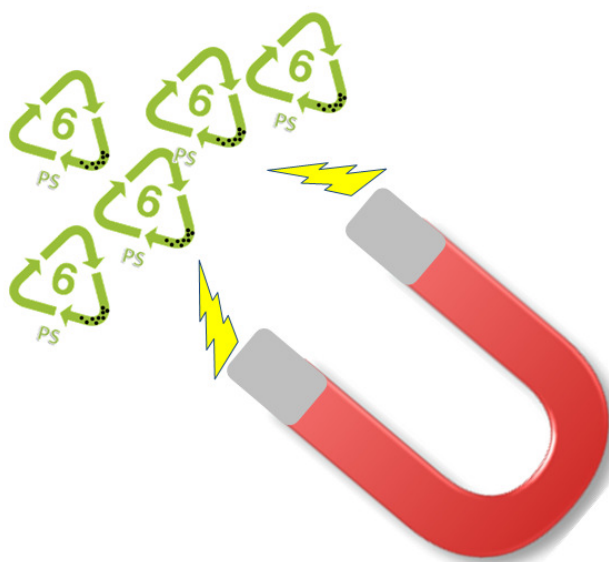
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The ubiquitous presence of plastics, an obvious consequence of their usefulness and low price, has turned them into a problem of environmental and safety concern. The new plastic economy, an initiative recently launched by the World Economic Forum and Ellen MacArthur Foundation, with analytical support from McKinsey & Company, promotes a change in the use of plastic worldwide around three main pillars: redesign, reusing and recycling. Recycled plastics, with the aim of extending their life span, can be used to synthesize materials for analytical purposes.

In this communication, polystyrene (PS) trays, previously used for food packaging, are proposed as polymeric source for the synthesis of magnetic nanocomposites. The synthesis plays with the solubility of PS in different solvents in such a way that PS is gelled in the presence of cobalt ferrite nanoparticles which are finally embedded in the polymeric network. The extraction capability of the magnetic PS nanocomposite was evaluated using the determination of four parabens (methylparaben, ethylparaben, propylparaben and butylparaben) in water using liquid chromatography-tandem mass spectrometry as model analytical problem.

Under the optimum conditions, limits of detection and quantitation were in the range of 0.05-0.15 and 0.15-0.5 ng/mL, respectively. The precisions, expressed as relative standard deviation (RSD), varied between 4.4 and 8.5% and the relative recoveries for analysis of the water samples were in the interval 81.2-104.5%.



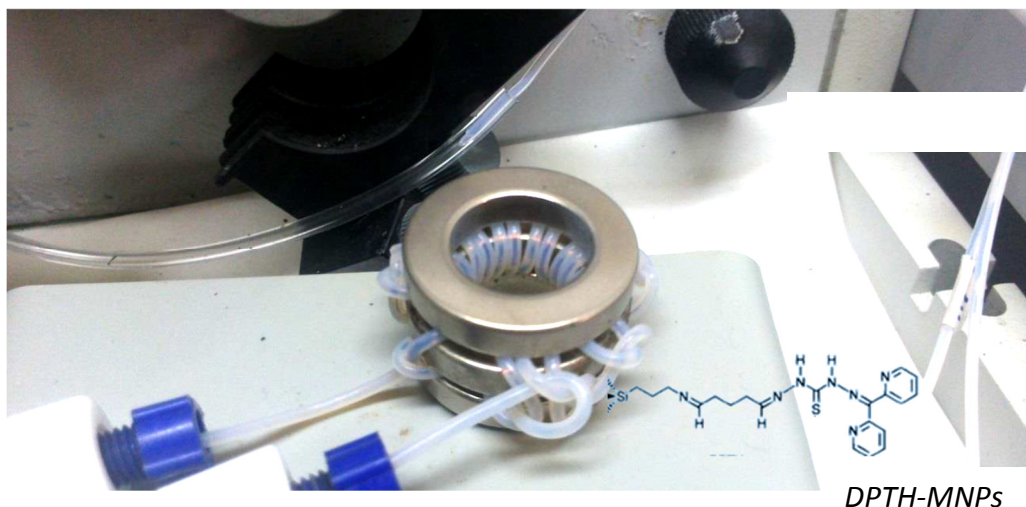
**SEQUENTIAL DETERMINATION OF TRACES OF As, Sb AND Hg BY ON-LINE MAGNETIC SOLID PHASE EXTRACTION COUPLED WITH HR-CS-CVG-GFAAS**

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A green and rapid method was developed for the simultaneous separation/preconcentration and sequential monitoring of arsenic, antimony and mercury by flow injection magnetic solid phase extraction coupled with on-line chemical vapor generation and determination by high resolution continuum source graphite furnace atomic absorption spectrometry. The system is based on chelating/cationic retention of the analytes onto a magnet based reactor designed to contain functionalized magnetic nanoparticles (MNPs). The MNP core allows overcoming the back-pressure problems that usually happen in SPME methods with NPs thanks to the possibility of immobilizing the MNPs by applying an external magnetic field. Several chemical and flow variables were considered as factors in the optimization process using central composite designs. With the optimized procedure the detection limits obtained were 0.2, 0.003 and 0.4  $\mu\text{g/L}$  for As, Sb and Hg respectively. For the quality control of the analytical performance and the validation of the developed method the analysis of two certified samples TM 24.3 and TMDA 54.4 Fortified Lake Waters was addressed. The results showed good agreement with the certified values.



# **SIMULTANEOUS DETERMINATION OF TRACES OF Pt, Pd, Os, Ir, Rh, Ag AND Au BY USING MAGNETIC NANOPARTICLES SOLID PHASE EXTRACTION COUPLED WITH ICP OES.**

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The direct analysis of these target analytes is very limited being essential sample pre-treatment techniques and the use of very sensitive instrumental techniques to carry out determinations. The inductively coupled plasma optical emission spectrometry shows a poor sensitivity because the concentration of some elements in environmental samples is below the detection limit of ICP OES. To solve this problem, preconcentration separation procedures have been proposed, minimizing the spectral and matrix interferences. Thus, enrichment is a very important issue for achievement of low detection limits [1-4].

In this study, a chelating resin 1,5 bis (di 2 pyridil) methylene thiocarbonohydrazide bonded to iron oxide magnetic nanoparticles (DPTH-MNPs) were synthesized. These magnetic nanoparticles were employed as a solid phase extraction (SPE) adsorbent for the separation and concentration of trace amounts of 7 elements (Au, Ag, Pd, Pt, Ir, Rh and Os) from environmental water samples. The main aim of this work was to develop a precise and accurate method for the simultaneous determination of the maximum possible number of elements by using this new absorbent and a multimode sample introduction system (MSIS). The MSIS acts as a system for the generation, separation and introduction of chemical vapours (CVG) and also as an introduction system for sample aerosols, in a simultaneous form, into an inductively coupled plasma-optical emission spectrometer. The on-line SPE-CVG-ICP-OES system developed was applied in the determination of the aforementioned metals in natural water samples (sea water, estuarine, lake and river water), with the least demanding and simple sample preparation procedure. The developed method was validated by analysing natural water certified reference materials (TMDA 54.4 fortified lake waters and SRM 1643e, trace elements in water; and National Institute of Standards and Technology (NIST), NIST-2557 autocatalyst). Sea water, tap water and well water samples collected from Malaga (Spain) were also analysed. The procedure has been demonstrated to be fast, easy, automatic, selective and economical, and the sensitivity was good.

The main advantage of DPTH-MNPs is its very good stability and resistance because chemisorption of chelating molecules on the surface of solid supports provides immobility, mechanical stability and insolubility. The precision (RSD), accuracy (by standard addition or recovery) and limit of detection (LOD) were used to evaluate the characteristics of the procedure. Furthermore, the proposed method was applied in the simultaneous determination of the 7 elements mentioned above with a sample throughput of about 13 h<sup>-1</sup>, thereby, reducing the time of analysis and the volume of reagents and sample required.

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## DETERMINATION OF TITANIUM DIOXIDE NANOPARTICLES IN COSMETICS

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'Nanomaterial' means an insoluble or biopersistent and intentionally manufactured material with one or more external dimensions, or an internal structure on the scale from 1 to 100 nm [1]. Recently, the use of nanoparticles (NPs) in the industry has increased drastically due to their different applications. NPs are used in cosmetics, in medicine (drug delivery and surgical material), in electronics, in the food industry (additives and food packaging), and in textiles.

Nowadays, titanium dioxide ( $\text{TiO}_2$ ) and zinc oxide ( $\text{ZnO}$ ) nanoparticles are used to modify physicochemical properties of cosmetics providing them protection against UVA and UVB radiations [2]. Therefore,  $\text{TiO}_2$  NPs and  $\text{ZnO}$  NPs replace UV filters now that their use are regulated or even in some cases forbidden. Before marketing a cosmetic modified with NPs, the size and physical and chemical properties of NPs must be reported to the European Commission [1].

NPs offer numerous advantages but there are very few studies on their possible toxicity. NPs in cosmetics can penetrate into the skin, specifically in the stratum corneum (SC) of the epidermis which is responsible for the barrier function of the skin [3]. Once the NPs have penetrated through the skin, they reach the bloodstream and they are accumulated in organs like heart, brain, liver, kidneys, and spleen.

Thus, analytical methods for characterizing and quantifying NPs in complex samples such as cosmetics should be developed.

In this communication, a pre-treatment method of cosmetic samples based on ultrasonication before  $\text{TiO}_2$  determination by Single Particle Inductively Coupled Plasma Mass Spectrometry (sp-ICP-MS) is proposed. Several experiments were performed by ultrasonication  $\text{TiO}_2$  NPs of 50-100 nm sizes to ensure  $\text{TiO}_2$  NPs stability after sample pre-treatment. The procedure has been validated (analytical recovery and precision), and has been applied to different moisturizing creams.

**Acknowledgement**

This research was supported by the Spanish Government (Ministerio de Economía y Competitividad, project CTQ2015-68094-C2-2-R). The authors belong to the Galician Competitive Research Group 6RC2014/2016. I. Rujido-Santos thanks Programa PRINCIPIA (Xunta de Galicia-GAIN) for a research contract. All these programs are co-funded by FEDER (UE).

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# FAST ANALYSIS OF CuONPs BY UV-Vis SPECTROPHOTOMETRY

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The work carried out takes advantage of the ability of diethyldithiocarbamate (DDTC) to form a complex with Copper(II) that shows an absorption band at 447 nm in aqueous medium. According to this, Copper(II) oxide nanoparticles (CuONPs) were subjected to the action of a powerful reducing agent ( $\text{NaBH}_4$ ) for the DDTC to combine with the resulting “reduced” CuONPs. This CuONPs-DDTC system also absorbs visible radiation at 447 nm.

As described above, one of the obvious problems to face was the possible interfering effect caused by Copper(II). Therefore, studies were done related to this interference: the effect of the direct addition of different reagents (EDTA, ammonia, acetylacetone, ...) to the Copper(II)-and-CuONPs-containing samples was investigated, with no success. Finally, another strategy was adopted and the addition of ammonia to the sample in order to remove the interference by Copper(II) led to the formation of the well-known  $[\text{Cu}(\text{NH}_3)_4]^{2+}$  ion, that exhibits an absorption maximum at ca. 600 nm. Thus, Copper(II) present in the sample could be quantified at that wavelength and its concentration subtracted from that obtained when, afterwards, the absorption of radiation at 447 nm was recorded to determine the Cu(II)-DDTC complex plus the “CuONPs-DDTC” associate. The method was validated and applied to the determination of CuONPs in aqueous samples.

**Acknowledgement:** this research was supported by the Spanish Government (Ministerio de Economía y Competitividad, project CTQ2015-68094-C2-2-R) and the Xunta de Galicia (Galician Competitive Research Group 6RC2014/2016).



**RUM CLASSIFICATION USING FINGERPRINTING ANALYSIS BY HEAD SPACE SOLID PHASE MICROEXTRACTION COUPLED TO GAS CHROMATOGRAPHY-MASS SPECTROMETRY**

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Nowadays, numerous methods have been described for the differentiation and classification of alcoholic beverages based on the analysis of the volatile composition [1]. With this aim, gas chromatography (GC) coupled to mass spectrometry (MS) is often used in combination with head space solid phase microextraction (HS-SPME). This combination has been applied to different matrices as wine or beer as well as to other popular spirit beverages, such as whiskey, vodka, gin, brandy or cognac. However, rum studies are less frequent, and the complex elaboration of this kind of beverage makes it a potential object of study, due to the wide variability and differences that may arise in its production. Moreover, beverage vendors have always used different categories to identify their rums and distinguish them from competitors, such as *Premium* or *Reserve*, which allow for a clear differentiation from other cheaper rums. In many cases, good and poor quality rums are included in these categories without any quantitative justification. Notwithstanding these classifications are not related to the rum age, fermentation, distillation, mixing or style, but there are marketing strategies to increase consumers' confidence. Due to the complexity and variability of rum composition, their classification represents an analytical challenge.

The aim of this study was the development of a comprehensive and robust analytical strategy for the analysis of the volatile/semivolatile compounds for rums differentiation and their quality classification. The analyses were carried out with commercially available rum samples from local liquor stores. After the HS-SPME-GC-MS analysis, the raw data were processed applying available statistical tools. For exploratory data analysis, unsupervised chemometric techniques as hierarchical cluster analysis (HCA) and principal component analysis (PCA) were applied. Meanwhile, supervised techniques as linear discriminant analysis (LDA) and *k*-nearest neighbors (*k*NN), were tested to train the classifier on the labelled examples and make predictions on the unlabelled data, achieving classification results due to the correlation between samples. All the chemometric tools employed allowed for the correct classification of the complete rum batch (a total of 33 brands rum of different ages). The HCA showed relevant differences between the rum samples due to addition of honey, syrup and manually flavoured. Therefore, these rum types were clearly classified by this chemometric method. PCA indicated 49 ions as relevant chemical descriptors related to discriminant compounds (e.g. hexanoic acid, octanoic acid, decanoic acid, ethyl acetate or 5-(hydroxymethyl) furfural) for the correct rum classification. On the other hand, when LDA was used the best results were obtained with the previously selected ions, allowing an analytical discrimination of the rum batch with a 98% accuracy. The *k*NN analysis was used as confirmation technique for the only doubtful result.

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## USO DE UN SENSOR RAMAN PORTÁTIL PARA LA DETECCIÓN DE FRAUDES EN JAMÓN IBÉRICO

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El cerdo Ibérico tiene una gran repercusión en la economía en España. Actualmente podemos encontrar cerdos ibéricos 100% así como cerdos derivados del cruzamiento genético de cerdos ibéricos con aquellos de la raza estadounidense Duroc, pudiendo etiquetarse éstos últimos también como cerdos ibéricos. Por otro lado, los cerdos ibéricos son alimentados bajos distintos regímenes, y por tanto sus productos derivados, entre los que se incluye el jamón, pueden ser clasificados en diferentes categorías –bellota o cebo- en función de la alimentación recibida. El jamón de bellota procede de cerdos ibéricos alimentados al aire libre con una dieta de bellota y hierba, mientras que el jamón de cebo procede de cerdos alimentados a base de pienso concentrado en granjas. Esto causa grandes diferencias en la calidad y el precio final del producto, implicando que el jamón ibérico sea susceptible de fraude. De hecho, el fraude en el etiquetado del jamón es un serio problema que causa grandes pérdidas económicas en el sector. A pesar de este problema, actualmente la legislación no contempla ningún método analítico para el control de la autenticidad del jamón ibérico.

La espectroscopia Raman es una herramienta con un gran potencial en el campo de la agroalimentación. Se caracteriza por ser una técnica vibracional no destructiva, rápida, capaz de proporcionar información química de la muestra en unos pocos segundos. Se han analizado mediante esta técnica, muestras de jamón de raza ibérica 100%, así como jamones procedentes de cerdos reproducidos mediante cruces genéticos con la raza Duroc (50-75% raza ibérica), todos ellos alimentados mediante bellota. Asimismo, se han analizado muestras de jamón ibérico (50% raza ibérica) de cebo. Visualmente no se apreciaron diferencias significativas en el espectro Raman de los mismos, por lo que los datos se procesaron usando técnicas quimiométricas basadas en el uso de la información contenida en todo el espectro. Los modelos quimiométricos fueron construidos utilizando el 80% de las muestras, dejando un 20% de las mismas para la posterior validación del mismo. Inicialmente, se llevó a cabo un análisis no supervisado por componentes principales (PCA) para reducir la dimensionalidad y extraer la información relevante. A continuación, se realizó un análisis discriminante lineal (LDA) para incorporar la información de la clase al modelo y comprobar si las muestras se agrupaban en conjuntos separados. Finalmente, se aplicó el método de k-NN para obtener el porcentaje de muestras clasificadas correctamente. Estudios preliminares han demostrado el potencial del sensor basado en espectroscopia Raman para la clasificación de muestras de jamones según la raza del cerdo y el régimen de alimentación.

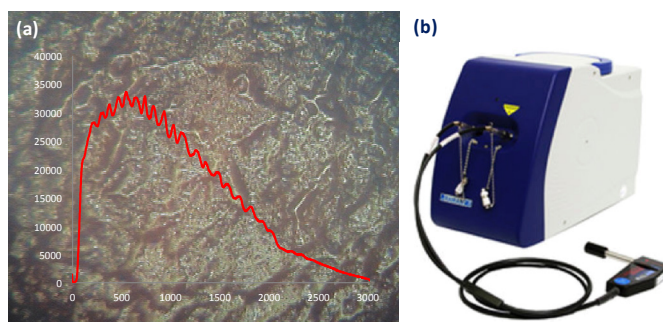


Figura 1. (a) Imagen de microscopio óptico de una muestra de jamón ibérico de cebo y espectro Raman de la misma. (b) Espectrómetro Raman portátil.

**FROM SIMPLER TO MORE COMPLEX CHEMOMETRIC APPROACHES FOR THE SPECTROPHOTOMETRIC DETERMINATION OF TARTRAZINE AND ALLURA RED IN FOOD SAMPLES****Ane Bordagaray, Rosa García-Arrona, Sergio Dávila, Miren Ostra, Maider Vidal**

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Synthetic additives are usually added to food in order to improve appearance, smell, flavor, color and texture. These compounds do not have nutritional value, but they are added to the food and drinks in minimal quantities with the aim to modify their sensory properties [1]. Azo colorants such as tartrazine (E102) and allura red (E129) are used to confer yellow and red color to food, drinks and cosmetics. The European Union regulates the authorized maximum levels for this kind of additives [2].

The quantification of this kind of analytes in foodstuff is usually performed by separation techniques, such as high performance liquid chromatography (HPLC) or electrophoresis (EC). Nevertheless, those techniques usually require relatively long times as well as the use of additional solvents and reagents.

The aim of this work is to develop a simple, fast and reliable procedure to determine tartrazine and allura red in different liquid food matrices with UV-VIS spectrometry and multivariate calibration methods such as Multiple Linear Regression (MLR), Partial Least Squares Regression (PLS) or Multivariate Curve Resolution – Alternating Least Squares (MCR-ALS). All of them have shown satisfactory results when applied to synthetic mixtures of both colorants, with concentrations ranging between 0.2 and 2 mgL<sup>-1</sup>. Relative prediction errors below 5% have been obtained in all cases. Good reproducibility and repeatability results have been obtained, being relative standard values lower than 3% for both analytes and no significant differences have been observed when comparing results to those obtained with HPLC.

The models have been applied to the determination colorants in food samples including liquid jellies, non-alcoholic beverages, ice-pops and other liquid and solid food colorants used in cookery. The performance of different algorithms in the presence of interferences has been compared and different strategies are proposed depending on the complexity of the analyzed samples, from the simplest approach applying MLR with Microsoft Excel ® to a more complex approach by using a specific toolbox in MatLab ® for MCR-ALS.

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## ENFOQUE QUIMIOMETRICO EN LA COMPARACIÓN DE ESPECTROS DE MASAS COMPLEJOS: ÍNDICES DE SIMILITUD

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La comparación y el tratamiento de espectros de masas complejos puede ser una tarea difícil de llevar a cabo. Entendemos como espectro de masas complejo aquel que posee numerosas señales, como por ejemplo el obtenido a partir de un digerido de péptidos procedente de una proteína (huella dactilar de peptídica). En numerosas ocasiones se pretende evaluar el cambio de estas señales a lo largo del tiempo lo que hace necesario que sean comparados diferentes espectros. Debido a la complejidad que presentan, detectar visualmente cambios entre ellos es muy difícil y puede llevar a errores.

Un enfoque válido y sencillo para detectar posibles cambios en espectros de masas complejos es el uso de índices de similitud. Se definen como: "*Quantity that describes the equivalence of two objects characterized by multivariate data*" (IUPAC Vocabulary of concepts and terms in chemometrics, 2016). Se basan en la comparación, uno a uno, de los elementos que conforman dos vectores. Esto implica que el espectro de masas tiene que ser transformado previamente en un vector de datos que contenga toda la información relevante del espectro. Los índices de similitud normalmente evalúan la distancia, la orientación espacial o la correlación matemática entre los elementos que conforman dos vectores, de manera que basándose en ellos, se puede inferir sobre el grado de similitud entre ellos. Han sido propuestos diferentes índices de similitud para la comparación de vectores, pero lo más usados son el coseno del ángulo determinado por ambos vectores en el espacio multidimensional (COS), el coeficiente de determinación entre los elementos de ambos vectores ( $R^2$ ) y los índices basados en la medida de la distancia entre los elementos de ambos vectores (p.e., BRAY-CURTIS). Los valores (absolutos) que adquieren varían entre 0 y 1, de manera que la similaridad será mayor cuanto más se acerque el valor a 1.

En esta comunicación se propone una metodología que permite tratar los espectros de masas complejos y adecuarlos para que puedan ser comparados mediante análisis de similaridad. Una vez obtenidos los vectores procedentes de cada espectro se calcula los índices COS,  $R^2$  y BRAY-CURTIS. Se presenta un nuevo índice denominado por nosotros como índice de cercanía ("nearness index", NEAR) basado en la proximidad espacial de dos vectores. Esta metodología se ha aplicado a un caso práctico: la comparación de huellas dactilares peptídicas para el estudio de estabilidad de anticuerpos monoclonales terapéuticos.

**UN NUEVO ENFOQUE PARA LA DESCRIPCIÓN DEL DESEMPEÑO DE MÉTODOS ANALÍTICOS DE CRIBADO BASADOS EN EL USO DE CLASIFICACIÓN MULTIVARIANTE**

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Tradicionalmente los métodos de cribado (o "screening") se consideran métodos de análisis cualitativo, independientemente de que la decisión se tome sobre una medida cuantitativa (la intensidad de una señal, un valor de concentración) o cualitativa (la aparición de un color u otra evidencia física). Cualquier método cualitativo se puede considerar un método de clasificación binaria, ya que el objetivo principal de tales métodos es decidir si una determinada muestra cumple o no una característica previamente establecida; en el caso de que la decisión sea positiva, generalmente ello implicaría la realización de un segundo análisis cuantitativo de confirmación.

Los métodos analíticos basados en el uso de quimiometría o, en nomenclatura más recientemente, de minería de datos han añadido una modalidad adicional a los métodos analíticos de cribado. En estos métodos, la decisión se toma después de un tratamiento adecuado que se aplica sobre un vector de datos analíticos representativo de la muestra, p.e., un conjunto de valores numéricos correspondientes a diferentes características o propiedades físico-químicas, o una señal analítica completa (un espectro o un cromatograma), que se corresponde con lo que ha venido a denominarse "huella digital instrumental".

Con este fin se aplican métodos de clasificación multivariante en cualquiera de sus modalidades (análisis discriminante, modelado de clases, redes neuronales, árboles de decisión, etc.). Tradicionalmente estos métodos se caracterizan a partir de dos indicadores que describen la calidad de las clasificaciones, como son la sensibilidad y la especificidad, que inciden en la probabilidad de acertar la clasificación considerando el total de muestras sometidas a análisis, y que se establecen en el estudio de validación del método.

Sin embargo, como demostraremos en esta comunicación, la información que suministran ambos indicadores es insuficiente y puede considerarse incluso como redundante. Por lo tanto es necesario calcular un indicador adicional, que en este contexto recibe también el nombre de precisión, y que informa sobre la probabilidad de acertar con relación al número de muestras que se clasifican como conformes (o no conformes). La garantía del usuario sobre los resultados del método de cribado se basará en: (i) los valores establecidos de precisión y sensibilidad (que también puede corresponderse con veracidad); y (ii) la distribución relativa (ocurrencia) de muestras conformes y no conformes.

Todos estos aspectos serán descritos en la comunicación, así como el uso de algunos indicadores de utilidad práctica para el usuario, como son el índice de conformidades, y la relación de muestras que deberán ser sometidas a análisis cuantitativo, y cuyos valores se derivan de los anteriores. Los valores de estos índices se derivan a partir de los valores anteriores.

## COMPARACIÓN ROBUSTA DE LAS PENDIENTES DE DOS RECTAS DE CALIBRADO CUANDO SE DISPONE DE POCOS PUNTOS EXPERIMENTALES EMPLEANDO UNA TÉCNICA BOOTSTRAP

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La comparación de las pendientes de las rectas de calibración (o estandarización) obtenidas empleando patrones acuosos y el método de las adiciones estándar (SAM) es un trabajo habitual en los laboratorios de análisis. Frecuentemente ambos protocolos implican muy pocos patrones de calibración y, en consecuencia, no siempre se dispone de las condiciones que garantizan la validez de los métodos estadísticos (por ejemplo, la aplicación del test *t* de Student). Se deduce, pues, que la toma de decisiones se puede ver profundamente afectada por estos problemas de base.

Existe, por tanto, la necesidad de utilizar métodos estadísticos robustos que no se vean distorsionados por el número tan reducido de patrones que habitualmente empleamos. En los últimos años se han presentado diversas estrategias no paramétricas, algunas de las cuales están basadas en *bootstrap*. De entre ellas se han elegido varias que habían sido desarrolladas originalmente para operar correctamente cuando se disponía de un número reducido de estándares, si bien el mínimo se había fijado en 20 [1-3], y se han estudiado con más profundidad para evaluar cómo funcionan en las situaciones habituales de laboratorio:

- (i) Número muy pequeño de estándares de calibración (entre 3 y 9).
- (ii) Existencia de homocedasticidad y heterocedasticidad (varianza creciente con la concentración).
- (iii) Diferentes distribuciones de los residuales del calibrado (normal, asimétrica y normal incluyendo un anómalo).
- (iv) Combinando esas condiciones se han modelado 144 escenarios diferentes.

Los resultados obtenidos se puede resumir como:

- (i) El test *t* de Student es la mejor opción sólo cuando los residuales son normales y homocedásticos, aunque esto es difícil de evaluar con muy pocos estándares de calibración.
- (ii) El método "*wild bootstrap*" conduce a porcentajes de rechazo próximos a los valores nominales (5% de falsos positivos), prácticamente en cualquier situación y es el que se recomienda en estos momentos. Se trata de un método no paramétrico y, por tanto, no es necesario estudiar en detalle la distribución de los residuales ni la existencia de anómalos (siempre que su número sea reducido).
- (iii) Se ha desarrollado un tutorial y un software de uso libre que puede ser utilizado por los laboratorios [4].

Agradecimientos: Programa de Consolidación y Estructuración de Unidades de Investigación Competitiva, Grupo QANAP (Ref. GRC2013-047) y proyecto CN2012/130, de la Xunta de Galicia. Proyecto MTM2014-52876-R del Ministerio de Economía y Competitividad.

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## NEAR INFRARED CHARACTERIZATION OF MARIJUANA SAMPLES

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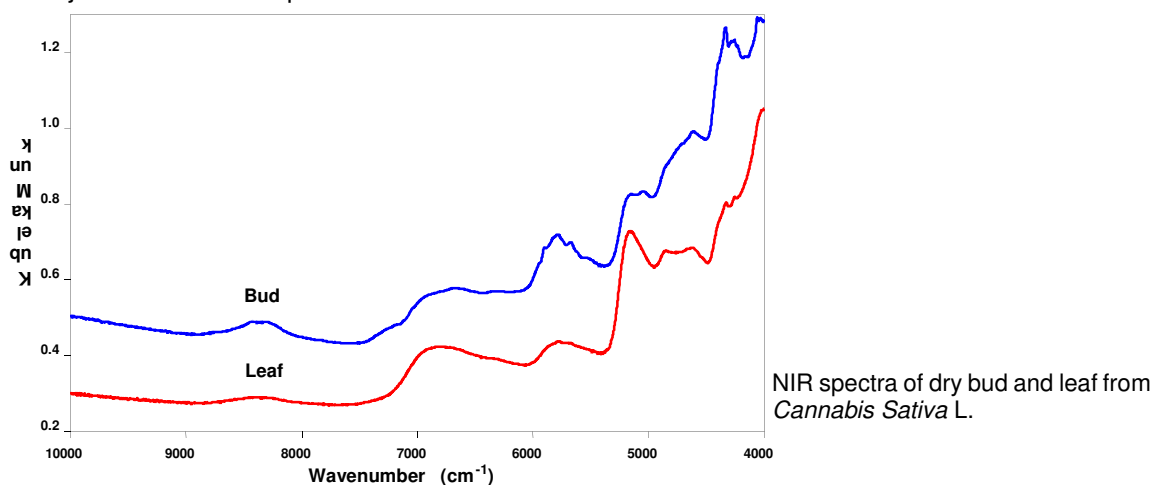
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Spanish rules regarding the production and commercialization of marijuana involves special penalties for seized amounts equal or higher than 10 kilograms. However, in the law it is indicated that this amount concerns dry weight of apical parts, thus avoiding the consideration of leaves and being focused only on buds. So, from the analytical point of view the challenges in marijuana analysis relate to the fast and accurate evaluation of humidity and nature of the seized plant parts more than on the THC content.

NIR spectroscopy provides fast and non-destructive measurements of solid samples without any previous sample preparation. The method can be highly accurate for water and other compounds determination if the concentration of analyte exceeds 0.1% of the total weight.

To determine the moisture of leaves and buds of *Cannabis Sativa* L., diffuse reflection spectra of an heterogeneous population of fresh leaves and buds was employed to evaluate the loss of weight as a function of time, the changes experienced during the process were reflected as a drop in the height of the peaks being possible to directly determined the water content using the band at  $6879\text{ cm}^{-1}$ . Results found evidenced that fresh leaves contain between 80.6 and 65.2% w/w of water while for buds content varies between 73.3 and 7.4% w/w, the last one corresponding to partially dried samples.

On the other hand, a new method has been developed for the direct determination of the percentage of leaves and buds of *Cannabis Sativa* L. in a milled mixture. It was made by using partial least-squares regression analysis of diffuse reflection near-infrared spectra of samples contained in a glass vials. From dried and crushed marijuana samples, eleven different mixtures of leaves and buds were employed to build the calibration model and to have a prediction. Multiplicative scatter correction (MSC) and mean center pre-processing of spectra and a wavenumber range from  $7528.8$  to  $3999.7\text{ cm}^{-1}$  were used. A root-mean-squared error of calibration (RMSEC) of 0.47% w/w, a root-mean-squared error of cross validation (RMSECV) of 2.5% w/w, and a root-mean-squared error of prediction (RMSEP) of 6.5% w/w were found, thus evidenced that diffuse reflection NIR spectroscopy is a good technique for characterization of marijuana mixture samples.

**Acknowledgements.**

Authors acknowledge the financial support of Ministerio de Economía y Competitividad-Feder (Project CTQ 2014-52841-P) and Generalitat Valenciana (Project PROMETEO II/2014/077).



**DETERMINACIÓN DEL NÚMERO DE COMPONENTES EN DATOS CROMATOCRÁFICOS Y CINÉTICOS MEDIANTE EL ESTUDIO DE LA CORRELACIÓN**J.J. Baeza Baeza<sup>1</sup>, F.F. Pérez Pla<sup>2</sup><sup>1</sup>Departament de Química Analítica, Universitat de València, c/Dr. Moliner 50, 46100 Burjassot<sup>2</sup>Institut de Ciencia dels Materials (ICMUV), c/Catedrático Beltrán 2, 46980, Paterna, Spaine-mail: [Juan.Baeza@uv.es](mailto:Juan.Baeza@uv.es)

Los métodos de análisis multivariantes son cada vez más importantes en Química y en otras áreas como Biología, Medicina o Economía. Así, es muy común, en Cromatografía y en Cinética Química, analizar señales bivariantes originadas durante el registro del espectro electromagnético de la disolución que se estudia, en función del tiempo.

Se han propuesto diversas metodologías para el análisis de datos multivariantes. Cabe destacar el Análisis de Componentes Principales, los Mínimos Cuadrados Alternantes, la Resolución Multivariante de Curvas o el Análisis de Componentes Independientes [1-2]. Su aplicación requiere la determinación del número de componentes (o factores) que permiten reconstruir la señal. Un ejemplo es el tratamiento de datos cromatográficos, pues a pesar de las mejoras en la eficacia, siempre cabe la posibilidad de no poder separar completamente analitos con propiedades muy similares. En este caso, la deconvolución de los picos solapados requiere conocer el número de especies que contribuyen a la señal. Otro campo de aplicación es el análisis de datos cinéticos mediante las ecuaciones de Draper-Box [3] que también requiere un conocimiento exacto del número de componentes.

Se han propuesto diversos métodos para determinar el número de factores que contribuyen a la información contenida en un conjunto de datos [4]. La determinación de los valores propios nulos del segundo momento o de los valores singulares de la matriz de datos son los más utilizados. No obstante, el número de valores propios/singulares mayores que cero suele ser superior al número de componentes que contribuyen a la información debido a la existencia de ruido en la señal.

En esta comunicación se desarrolla un procedimiento simple para evaluar el número de componentes significativos en una matriz de datos bilineal. El método se basa en la extracción por etapas de la información y en la evaluación de la correlación entre los datos. Se proponen nuevos criterios para la selección de factores basados en el estudio de la información remanente y en su relación con el ruido.

Finalmente, se comparan los algoritmos propuestos con diversos métodos existentes aplicados a datos cinéticos y cromatográficos de diferente complejidad. Dentro de los estudios cinéticos, se realiza un análisis de la influencia de diferentes mecanismos de reacción en la dificultad de determinar el número de especies implicadas. Además, se compara la capacidad de detección de estados intermedios con diferente reactividad y contribución final a la señal.

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## AUTHENTICATION OF TEA TREE ESSENTIAL OIL BY INFRARED SPECTROMETRY

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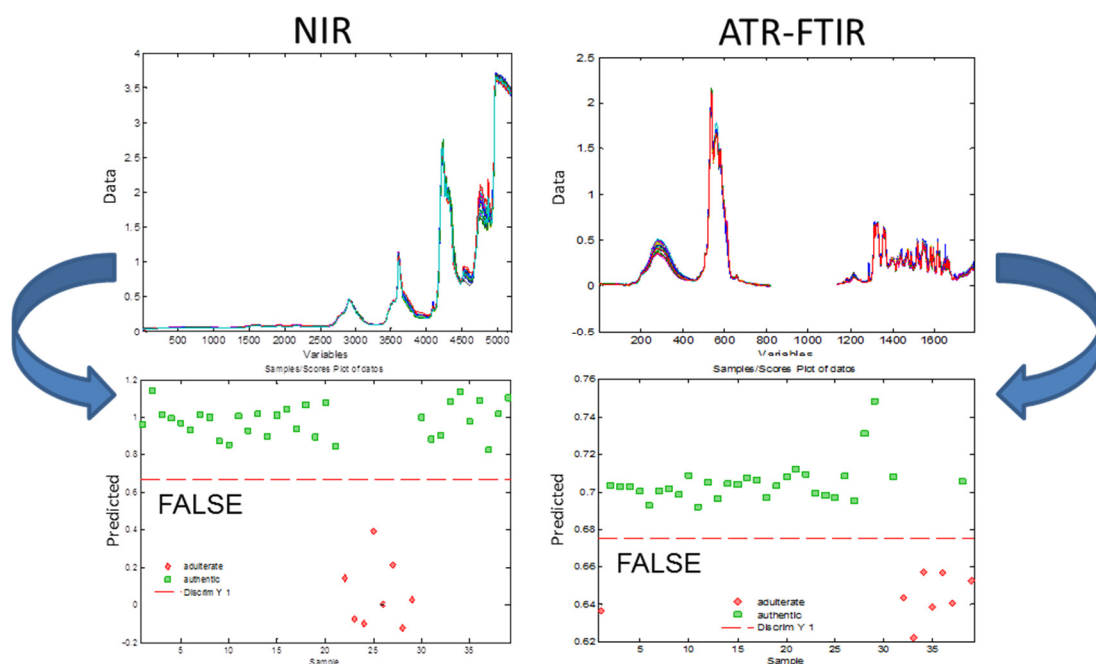
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The benefits and potential of 100% pure Australian tea tree oil, obtained from *Melaleuca alternifolia*, have been recognised for more than 70 years. Tea tree oil is a natural essential oil and natural antiseptic, world renowned for its purity and quality, and is widely formulated into many cosmetic and personal care products.

A new procedure, alternative to chromatography technique, has been developed for the authentication of tea tree oil commercial samples. The method is based on NIR and ATR-FTIR measurements followed by chemometric treatment. A set of 38 samples, including adulterated and non-adulterated Australian labelled tea tree oil samples, obtained around the world were used. Several models were built through the evaluation of the wavenumber range, data pre-processing and election of appropriate number of latent variables, using the samples as a calibration set to develop PLSDA model. Preliminary models were developed by using a calibration set of 30 samples and a validation set of 8 samples.

From NIR spectra, a PLSDA model, using the orthogonal signal correction (OSC) pretreatment of 14000-4500  $\text{cm}^{-1}$  spectral region with 3 latent variables, was selected to discriminate between authentic and adulterate samples using the leave one out cross validation. Model was characterized by a RMSEC and RMSECV values of 0.111 and 0.282 respectively. The same data pretreatment was employed to develop the model from ATR-MIR spectra. In this case, model included the 4000-2420 and 1815-550  $\text{cm}^{-1}$  spectral region using 1 latent variable. The RMSEC and RMSECV values in this case were 0.397 and 0.42, respectively. In both cases, PLSDA models allowed a clearly discrimination between adulterated and non-adulterated samples, as it shows **Figure 1**.

A set of more than 200 new samples, including adulterated and non-adulterated products, are nowadays used to evaluate the initial models and improve their performance.



**Figure 1.** NIR and ATR-FTIR spectra of analyzed samples and the classification of samples into adulterated and non-adulterated Australian tea tree oil.

**DEVELOPMENT OF ROBUST NIR CALIBRATION MODELS FOR THE MONITORING OF PHARMACEUTICAL TABLET COMPACTION AND WET GRANULATION****A. Miró, M. Alcalà**

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The quantification of active principle ingredient (API) throughout the whole production process is one of the most important challenges during pharmaceutical manufacturing. Using near infrared (NIR) spectroscopy combined with chemometrics for this purpose is nowadays a widespread practice. In fact, it is included as part of the process analytical technology (PAT) framework, described by the regulations agencies in the area. The robustness is a desired characteristic in this kind of process analysis due to the costs of the new instrument installation and calibration models development. The design, preparation and selection of calibration sets for pharmaceutical analysis are critical factors to obtain a robust calibration model. The significant variation sources of the final application need to be considered during calibration to provide a successful predictive ability. There are a number of different strategies to provide solution to this issue: laboratory, industrial and pilot plant samples, combined calibration sets, data pre-treatment, among others.

The *process spectrum*<sup>1</sup> is a methodology for tuning calibration data sets including the changes due to diverse stages of a manufacturing process by mean of an algebraic procedure. It has been successfully applied to the incorporation of the variability caused by chemical and physical changes during pharmaceutical processes to the near infrared (NIR) spectra of laboratory samples. Typically, this strategy has been useful to calculate the process contributions to the NIR spectra in the concentration centroid, however the properties of this methodology in extreme points of the concentration ranges has not been studied yet. With the aim of evaluating such behavior, as well as its properties in function of chemical and physical changes, 350 laboratory samples has been prepared. This total amount is the sum of 35 powder samples, 35 wet granulated samples, 140 tablets prepared from powder and 140 tablets prepared from granulated. 4 tablets per sample were prepared to include the variability of compaction pressure. All the samples sets covered the nominal range of API from 7 to 13 % w/w combined with the use of placebos spanning the concentration values of the excipients around relative  $\pm 5\%$  of the nominal formulation. Experimental design for sample preparation was used to minimize collinearity between concentrations. 2 and 3 spectra replicate were acquired to include instrument variability. Resulting NIR data allows the evaluation of the spectral changes due to pharmaceutical process stages and provides relevant information to develop more robust calibration models for API quantification. Calibration improvement is evaluated comparing the classical strategy of process spectrum with diverse spectral pre-treatments among other approaches.

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## APROXIMACIÓN METABOLÓMICA PARA IDENTIFICAR EN PLASMA FIRMAS ESPECTROSCÓPICAS BASADAS EN RMN ASOCIADAS A DISTINTOS ESTADIOS CLÍNICOS DE LA ENFERMEDAD DE PARKINSON

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La enfermedad de Parkinson (EP) ocupa el segundo puesto mundial en incidencia de trastornos neurodegenerativos, tras la enfermedad de Alzheimer. En un futuro cercano su importancia aumentará, debido a los cambios en la estructura etaria de la población [1]. El diagnóstico de la EP continúa siendo clínico, siendo posible la confirmación del diagnóstico únicamente mediante un análisis histológico post-mortem [2]. Los problemas asociados a su diagnóstico se agravan más considerando que otras patologías pueden imitar los síntomas iniciales de la EP, retrasando el mismo. Hasta la fecha, no existen marcadores biológicos definitivos para realizar un diagnóstico diferencial preciso. Urge la necesidad de identificar biomarcadores sensibles y específicos que permitan detectar precozmente la EP, evaluar su severidad, pronosticar su curso y valorar la eficacia de los tratamientos.

Un biomarcador de la EP basado en muestras sanguíneas resultaría ideal dada la accesibilidad, mínima invasividad y bajo coste de una flebotomía. En los últimos años, se han producido avances alentadores en este campo [3-4]. No obstante, estudios de verificación más profundos son necesarios para confirmar y validar la precisión diagnóstica de los biomarcadores propuestos. Con todo ello, actualmente no se dispone de biomarcadores de uso clínico, capaces de anticipar la aparición de la EP o de constituir una prueba diagnóstica definitiva.

En este contexto, la estrategia metabolómica propuesta se basa en el uso combinado de la espectroscopia de resonancia magnética nuclear (RMN) y de potentes herramientas de análisis multivariado de los datos sobre muestras de plasma con el fin de investigar la existencia de patrones diferenciales (firmas metabólicas basadas en RMN) para pacientes de Parkinson en diversos estadios de la enfermedad frente al grupo de control de individuos sanos y a pacientes con otras patologías relacionadas (demencias no parkinsonianas). La etapa de pre-procesado de los datos (incluyendo la corrección de línea base y el alineamiento y compresión de las señales de RMN) se afrontó con especial cuidado debido a su complejidad y a la importancia crucial que su eficacia puede desempeñar en el éxito o fracaso final de la aproximación de clasificación a abordar. Sobre los espectros RMN corregidos se aplicó un potente método de selección de variables (*stepwise orthogonalization of predictors* – SELECT) con el fin de extraer un reducido subconjunto de variables discriminantes (RMN *fingerprints* reducidas) a emplear en el desarrollo de reglas de clasificación basadas en el Análisis Discriminante Lineal (*Linear Discriminant Analysis*, LDA) destinadas a conseguir la estratificación de la cohorte de pacientes estudiada. Así, mediante un sistema de clasificación secuencial en dos etapas se pudo discriminar, en primera instancia, entre pacientes de Parkinson, demencias no parkinsonianas y sujetos sanos de control (en base a una firma RMN formada por solo 30 variables y con un 100% y un 97.8% de asignaciones correctas en clasificación y validación cruzada, respectivamente), para posteriormente lograr la diferenciación inequívoca entre pacientes con Parkinson en estado inicial y avanzado (con signos de demencia) únicamente a partir de 14 variables. El objetivo final de las fiables clasificaciones propuestas es que estrategias similares a las que han propiciado su desarrollo puedan servir como herramientas de detección y diagnóstico rápidas, simples y sensibles, empleando las firmas metabólicas reducidas basadas en RMN de nuevos pacientes como única entrada al sistema.

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## GLUTEN DETERMINATION BY IMMUNOASSAY USING ULTRASOUND-ASSISTED EXTRACTION AND NATURAL DEEP EUTECTIC SOLVENTS

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Gluten involves different proteins, named as prolamins and glutelins, insoluble in water and 0.5 M sodium chloride. In addition, there is significant protein variability due to the cereal of origin and the food processing that can change protein structure and so their solubility [1], making it extremely difficult the determination of gluten in food. Enzyme-Linked ImmunoSorbent Assay (ELISA)-based methods are the most commonly used for this purpose. In general, ELISA allows to determine monomeric prolamins, in which the toxic fraction for gluten-sensitive people is located. In general, extraction of gluten is a limiting step in terms of effectiveness when a processed food is analysed. To that end, different ethanol-water solutions with a reducing agent such as 2-mercaptoethanol are used as extractant in order to prevent protein aggregation by disulphide bonds. Nevertheless, these mixtures can interfere in the antigen-antibody interaction [2] and even solubilise polymeric glutelins [1].

Natural deep eutectic solvents (NADESs) have recently been proposed for protein solubilization as an alternative to traditional solvents. NADESs, composed of natural primary metabolites such as organic acids, amino acids, sugars, urea etc., could explain the solubilization of proteins that normally are poorly soluble in the aqueous environment of cells [3]. On the other hand, gluten extraction involves heating and stirring (about 2 h). Ultrasound (US) has proved to be an effective energy for solid-liquid extraction since it improves the penetration of solvents into the cells and mass transfer, hence speeding up lysis, extraction and solubilisation processes. Some characteristics of NADESs, in special their high viscosity, could have a strong influence on ultrasound-assisted extraction [4].

In this work, fourteen NADESs were prepared using two natural primary metabolites and water. Viscosity of NADESs and gluten solubilisation in these solvents were studied. For comparison purposes, ethanol-water solutions were used. In order to speed up gluten solubilisation, the effect of dilution, temperature and sonication by a cup-horn sonoreactor was evaluated. Finally, six Eppendorf vials with 0.025 g of homogenized sample and 1 mL of NADES (20% v/v fructose-citric acid) were sonicated simultaneously for 15 min at 40% ultrasonic amplitude sonication. Electrophoresis and molecular fluorescence were used for characterizing of solubilised gluten. After centrifugation, the obtained extracts can be directly applied in the microplate. A reassessing of immunoassay system was necessary since kit solvents were replaced by the NADES. The citric acid with antioxidant character allow the elimination of the classical reducing agent. Different samples with and without gluten were analyzed. Recovery studies at two levels, *i.e.*, 20 and 80 ppm, were carried out. Recoveries were in the range of 79–106% with a relative standard deviation better than 15%. In addition, the direct application of extracts improves sensitivity by a factor of 10 as compared to the conventional use of the kit. A high sample throughput was also reached [5].

*Acknowledgements:* Financial support from the Spanish Ministry of Economy and Competitiveness (Projects CTQ2012-32788 and CTQ2015-68146-P) (MINECO/FEDER) is gratefully acknowledged.

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## SÍNTESIS Y CARACTERIZACIÓN DE DISOLVENTES SUPRAMOLECULARES CONSTITUIDOS POR AGREGADOS DE MICELAS OLIGOMÉRICAS DE ÁCIDO UNDECENOICO: APLICACIÓN EN PROCESOS EXTRACTIVOS

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SUPRAS (disolvente supramolecular) es un término relativamente reciente que hace referencia a la fase rica en tensioactivos (coacervado) separada de disoluciones coloidales mediante procesos de autoensamblaje. Este término enfatiza cómo los anfifilos forman estructuras supramoleculares autoorganizadas en la fase líquida separada, siendo esta la característica más distintiva en comparación con los disolventes moleculares y los líquidos iónicos. Una de las mayores desventajas en el uso de SUPRASs en química analítica es la pérdida de anfifilo que se produce en el tratamiento de muestras de elevado volumen, ya que el anfifilo se encuentra en equilibrio con la fase acuosa -a la concentración micelar crítica (CMC)- redundando este hecho en recuperaciones no cuantitativas. Por otro lado, la mayoría de los SUPRAS son incompatibles con cromatografía de gases, dada la elevada concentración de tensioactivo que se volatiliza. En este trabajo se sintetizaron y caracterizaron SUPRASs basados en oligómeros de ácido undecenoico para los que se ha propuesto en el pasado que su CMC es nula o, al menos, despreciable y que presentan elevado punto de ebullición.

Los SUPRASs aquí estudiados se sintetizaron añadiendo agua a disoluciones de oligómeros de ácido undecenoico (P.UDA) en tetrahidrofurano (THF). El agua promovió el autoensamblaje del oligómero y la separación de fases líquidas (Fig. 1). Se obtuvo así el respectivo diagrama de fases a partir de mezclas ternarias de P.UDA/THF/agua. Adicionalmente, se investigó la influencia de la temperatura y de la concentración de sales en estos límites y se determinó la ecuación empírica que relaciona el volumen de SUPRAS obtenido con la cantidad de anfifilo y agua utilizados en la síntesis. Finalmente, se evaluó la composición del SUPRAS bajo diferentes condiciones sintéticas y la organización nanoestructural mediante la técnica Cryo-SEM (Fig. 1).

Los estudios realizados demostraron que la composición global del disolvente y el tamaño de las gotitas de coacervado que lo forman pueden modificarse controlando el ambiente en el que se produce el autoensamblaje. Así, los SUPRASs caracterizados en este trabajo son altamente adaptativos, pudiendo revertirse sus características mediante modificación del modificando el entorno. En todo caso, el autoensamblaje espontáneo de estos disolventes siguió rutas predecibles, y su composición y volumen pueden preverse con precisión a partir de ecuaciones empíricas. Las propiedades descritas que presenta este tipo de SUPRAS los hacen sumamente atractivos para la extracción de analitos mediante cromatografía de gases acoplada a espacio de cabeza: gracias a la incorporación cuantitativa del anfifilo las extracciones resultan altamente eficientes en un gran intervalo de condiciones iniciales y, a diferencia de otros SUPRAS caracterizados anteriormente, su baja volatilidad resulta en cromatogramas HS-GC libres de interferencias generadas por el propio SUPRAS.

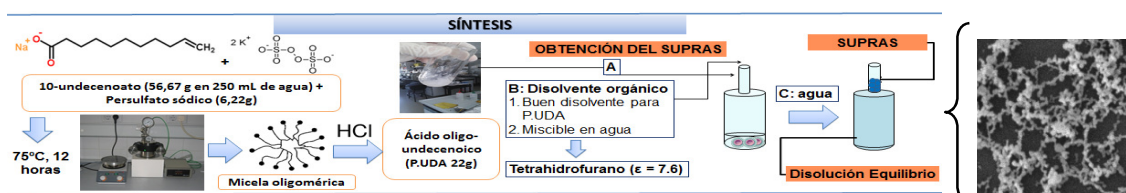


Fig.1: Síntesis del SUPRAS basado en P.UDA:THF:H<sub>2</sub>O y microfotografía Cryo-SEM

**Agradecimientos:** Los autores agradecen el apoyo financiero del MINECO (Proyecto CTQ2014-53539-R) y FEDER. J.A.S.D. y D.G.G. reconoce al MINECO por una beca de postgrado (FPU13/03796) y postdoctoral (FJCI-2014-20052), respectivamente.

## GREEN PROTEIN EXTRACTION WITH SULPHONATE-TERMINATED CARBOSILANE DENDRONS COATED NANOTUBES

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Protein sample preparation is required in many applications as those focused to obtain bioactive peptides, to quantitate proteins, to assess food quality, in proteomic analysis, etc. Most common methods for protein extraction require the use of high volumes of solvents as phenol or acetone [1,2]. For that reason, the development of new approaches satisfying the green chemistry philosophy is a challenge to overcome. In this sense, carbosilane dendrimers have demonstrated a great potential in protein sample preparation [3,4] and, for that reason, sulphonate-terminated carbosilane dendrons coated single-walled carbon nanotubes (SWCNTs) are a green alternative to usual methods employed in this field.

The present work is devoted to the study of the potential of dendronized SWCNTs in protein extraction evaluating the influence of pH conditions, dendron concentration and generation, and proteins nature. The interaction between three standard proteins (BSA, lysozyme and myoglobin) and dendronized SWCNTs was confirmed by SEM images and by the monitoring of the fluorescence of proteins. Protein-nanosystem interactions were promoted by neutral pH conditions and high dendron generation. The attachment of dendrons to nanotubes made possible their application in protein sample preparation comparing with corresponding dendrimers. In fact, dendron coated nanotubes were efficiently applied to the extraction of proteins from a complex fruit sample. Optimized conditions for nanosystem extraction method provided similar results to those obtained when using a traditional and non-environmentally friendly method. Moreover, it was demonstrated that the proposed method was compatible with the protein hydrolysis with a protease, yielding degrees of hydrolysis similar to those obtained when employing traditional extraction methods.

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## **DESARROLLO DE UNA PLATAFORMA UNIVERSAL PARA LA ELIMINACIÓN DE EFECTOS MATRIZ EN EL ANÁLISIS DE MUESTRAS BIOLÓGICAS MEDIANTE CROMATOGRAFÍA DE LÍQUIDOS Y ESPECTROMETRÍA DE MASAS**

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Uno de los principales retos de la aplicación de cromatografía de líquidos y espectrometría de masas (LC-MS) en bioanálisis, especialmente cuando se utilizan fuentes de electrospray (ESI), es la eliminación de los efectos matriz producidos por sustancias endógenas tales como fosfolípidos, proteínas y polisacáridos. La eliminación de proteínas mediante precipitación o exclusión en materiales de acceso restringido se realiza con éxito de forma rutinaria en bioanálisis. Sin embargo, dado el carácter anfifílico de los fosfolípidos, ninguno de los tratamientos generalmente aplicados a muestras biológicas (ej. extracción líquido-líquido o extracción en fase sólida) son eficaces en su eliminación.

Los fosfolípidos se acumulan en la superficie de las gotas de electrospray y originan la supresión de la ionización en LC-ESI-MS de los analitos con los que co-eluye debido a que inhiben la liberación de éstos a la fase gaseosa. Por otro lado, se adsorben fuertemente a la fase estacionaria de LC y producen cambios en los tiempos de retención de los analitos, incrementos en la línea de base del cromatograma y curvas de calibración divergentes.

En este trabajo se propone una estrategia general para la eliminación de proteínas, polisacáridos y fosfolípidos en bioanálisis, basada en el uso de disolventes supramoleculares volátiles con propiedades de acceso restringido (RAM-VOL-SUPRAS). El disolvente se sintetiza in situ, mediante procesos espontáneos de autoensamblaje y coacervación, al añadir hexanol y tetrahidrofurano a la muestra biológica. Las propiedades RAM del SUPRAS permiten la exclusión de proteínas y polisacáridos mediante mecanismos químicos y físicos, respectivamente. Los fosfolípidos se extraen en el SUPRAS y, una vez evaporado el extracto, permanecen en el residuo cuando éste se reconstituye con un disolvente apropiado. La capacidad de eliminación de interferentes, junto con la elevada eficacia de extracción del SUPRAS, permiten la integración de la etapa de extracción de compuestos en un amplio intervalo de polaridad y la purificación de la muestra.

La estrategia descrita se ha aplicado al tratamiento de muestra en la determinación de 13 bisfenoles y sus derivados en saliva humana mediante LC-ESI-MS/MS. La saliva es una muestra biológica no invasiva, ampliamente utilizada en el control de drogas y cuyo uso en estudios epidemiológicos para determinar la exposición humana a contaminantes está adquiriendo cada vez mayor importancia. El procedimiento consiste en la adición de hexanol (45  $\mu\text{L}$ ) y tetrahidrofurano (450  $\mu\text{L}$ ) a la muestra de saliva (1005  $\mu\text{L}$ ) y después de agitar y centrifugar la mezcla, se evapora el extracto de SUPRAS y el residuo se reconstituye con 300  $\mu\text{L}$  de agua: metanol (50:50, v:v). Los límites de detección (4 - 32  $\text{ng}\cdot\text{L}^{-1}$ ) y cuantificación (11 - 48  $\text{ng}\cdot\text{L}^{-1}$ ) alcanzados para los 13 bisfenoles son muy bajos y las recuperaciones de los mismos se encuentran en el intervalo del 83% al 105%. El método desarrollado, una vez validado, se ha aplicado a una población de un número restringido de individuos, seleccionados al azar, para conocer la concentración basal de bisfenoles y derivados y cómo varía dicha concentración tras la ingesta de determinados alimentos y tras fumar tabaco. En todos los casos, las concentraciones halladas se encuentran por debajo de los 2  $\mu\text{g}\cdot\text{L}^{-1}$ .

Agradecimientos: Los autores agradecen al MINECO (CTQ2014-53539-R) y a los Fondos FEDER la financiación recibida. Encarnación Romera-García agradece al MECO la beca FPU15/03704.

## APPLICATION OF HOLLOW FIBER LIQUID PHASE MICROEXTRACTION (HF-LPME) FOR DETERMINATION OF BIOACTIVE COMPOUNDS IN BERRIES

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Berries fruits such as raspberry (*Rubus × idaeus*), blueberry (*Vaccinium × corymbosum*) and strawberry (*Fragaria × ananassa*) are a rich source of phenolic compounds which have been claimed to have beneficial effects on human health due to their antioxidant activity, inhibition of low-density lipoproteins, decrease of cardiovascular diseases and some types of cancer [1]. The predominant bioactive compounds found in berries are anthocyanins, being about 50% to the quantified phenolic compounds, flavonols and phenolic acids. The use of hollow fibre liquid-phase microextraction (HF-LPME) is an attractive technique due to its simplicity, analytical precision, low consumption of organic solvents, low cost, absence of sample carryover, high enrichment factors, high throughput and facility for automation and conversion into a green analytical technique that has been applied to the determination of flavonoids and phenolic acids in plants and fruits [2]. For this purpose an analytical approach based on HF-LPME and ultra-high-performance liquid chromatography spectrometry (UPLC) has been performed.

The fruit samples were lyophilized, cryohomogenized and kept at -80°C until sample preparation. Phenolic compounds were pre-extracted for 30 min and heating was carried out by addition of 15 mL of 2M HCl and 1.5 g·L<sup>-1</sup> of butyl-iso-hydroxyanisol (BHA) solution in methanol to 0.5 g of sample powder. The extract was filtered by 0.2 µm Nylon membrane filter. Quantification of the analytes in the samples was performed by standard addition analysis. A 5-mL sample solution was introduced in a 10 mL glass vial and placed in a water bath under magnetic stirring. The hollow fiber (Accurel Q3/2 polypropylene, 600 i.d., 200 µm wall thickness and 0.2 µm pore size) was cut into 10 cm segments and was sonicated in acetone for 5 min to remove possible contaminants. Then, hexyl acetate (organic solvent) was then introduced into the fiber and immersed into the organic solvent for 30 s to impregnate their pores. The hollow fiber was bent to a U-shape and introduced into the sample vial following the sample solution stirring at 1000 rpm during the extraction. After 80 mins of extraction the acceptor solution was retracted with a syringe, transferred into a new vial. Finally, the solution was injected into the UPLC system. Separation and identification of phenolic compounds were performed using an Accela UPLC chromatograph (Thermo Scientific) equipped with a UV-Vis diode-array detector on a C<sub>18</sub> (250 × 4.6 mm, 5 µm particle size) column from Waters (Milford, MA, USA). Mobile phase A consist 0.1% (v/v) formic acid in water and mobile phase B acetonitrile acidified with 0.1% (v/v) formic acid.

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## EFFECT OF SAMPLE PRETREATMENT AND EXTRACTION METHODS ON THE DETERMINATION OF FLAVONOIDS FROM LEMON (*CITRUS LIMON*)

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Flavonoids have shown multiple beneficial effects on human health, being appreciated by both food and pharmaceutical industries<sup>[1]</sup>. Citrus fruits are a key source of flavonoids, thus promoting studies to obtain them. A study on the key role of lemon extraction methods and sample pretreatment on the analytical results is here presented. The objective of the study was to establish the differences between the most common both extraction —ultrasound-assisted extraction (USAE), microwave-assisted extraction (MAE), superheated liquid extraction (SHLE) and shaking extraction (SE)— and sample pretreatment methods (lyophilization, air-drying and no treatment —fresh sample) used for extraction of flavonoids from citrus fruits. The extraction conditions for USAE, MAE and SHLE were previously established based on a multiobjective optimization by a desirability model, which was used to maximize, at the same time, the concentration of 5 selected flavonoids in a HPLC-UV analysis<sup>[2]</sup>. Once determined the conditions for USAE (5.1 min; 60.3% of ethanol in water; 70% amplitude; 0.8 s/s duty cycle: 0.65 estimated desirability), MAE (12.6 min; 68.4% of ethanol in water; 171.4 W: 0.51 estimated desirability) and SHLE, (15 min; 73% of ethanol in water; 150°C: 0.66 estimated desirability) new extracts were obtained in suited conditions for each extraction method and used to compare them by LC–QTOF MS/MS analysis. Based on MS/MS information, the tentative identification of 32 flavonoids (9 flavanones, 14 flavones and 9 flavanols), which constituted the data set, was carried out. An unsupervised analysis by PCA, consistent with the ANOVA test, revealed a clear discrimination between extraction methods. Also, Tukey HSD ( $p \leq 0.01$ ) showed that only 7 out of 32 flavonoids were significantly different in the pairwise comparison of USAE and MAE —the most similar pair—, being the flavones (5 out of 7) the subclass that presented more significant differences. It must be emphasized that SHLE provided the extracts with the lowest concentration of flavonoids: 23 flavonoids were significantly more concentrated in SE extracts; 30 in those from USAE and 31 in MAE extracts. In general, the USAE method was the best to extract flavonoids, showing yields higher than all other methods in a shorter time. To evaluate the effect of sample pretreatment, lyophilized, air-dried (45 °C) and fresh samples were extracted by the USAE method. The PCA showed a clear discrimination among samples, which were also studied by ANOVA and pairwise mean comparison by Tukey HSD ( $p \leq 0.01$ ), thus revealing that flavanones were in general significantly more concentrated in extracts from lyophilized samples. On the contrary, and in contrast with the common assumption that lyophilization is the best pretreatment method, flavanols like quercetin derivatives were significantly more concentrated in extracts from air-dried samples. Considering the flavonoids pathway, these results suggest that polyphenol oxidase increases its activity in air-dried samples until the water activity is sufficiently low to inactivate the enzyme<sup>[3]</sup>. Finally, extracts from fresh samples provide a lowest concentration of flavonoids, thus demonstrating the suitability of dehydration prior to extraction of this class of compounds.

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## FORCHLORFENURON DETERMINATION IN FRUITS BY IMMUNOAFFINITY PRECONCENTRATION AND ION MOBILITY SPECTROMETRY

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Forchlorfenuron (N-(2-chloro-4-pyridyl)-N'-phenylurea (CPPU) is a synthetic phenylurea plant growth regulator [1,2]. In recent years, CPPU has been registered in many countries and is widely used in agriculture [3]. However, several studies showed that CPPU could be endocrine disruptor and had certain genotoxicity or ecotoxicity [4]. As a result, the maximum residues of CPPU in fruits and vegetables were strictly limited to less than 50 µg kg<sup>-1</sup> in China, Japan, European Union and other countries. Therefore, the rapid determination of CPPU in fruits and vegetables is of great significance.

Ion mobility spectrometry (IMS) is a gas phase ion separation technique at ambient pressure, in which ions are separated according to their individual velocities as they drift through an inert gas driven by an electric field. For a typical IMS device, the measured number of theoretical plates rarely exceeded 5000. Therefore, the incomplete resolution of peaks is a common situation in the analysis of complex samples by IMS. In recent years, the selectivity of sample treatments has been improved by using analyte-selective supports, such as immunoaffinity columns (IAC) [5].

The aim of this study is to highlight the advantages of IAC-IMS coupling in terms of sensibility, selectivity, accuracy and speediness of analysis using as example the determination of CPPU in fruits and vegetables. The analyte binding capacity of different IACs prepared from 0.5 g of antibodies (s3#22, p2#21 and p6#41) [6] were 251, 1166 and 970 ng CPPU, respectively. Additionally, the column reusability was evaluated using the analyte binding capacity as critical factor. IACs were repeatedly used (n = 10) as described above, and a continuous reduction in the binding capacity was observed. After 10 uses, the binding capacity values for CPPU were 40 and 60% of the original values for p2#21 and p6#41, respectively. Thus, p6#41 was selected as the most appropriate IAC column for further analysis.

The IACs have been evaluated in terms of immunosorbent binding capacity, optimum elution conditions, and reusability. IAC column was loaded with 1 mL of 400 µg L<sup>-1</sup> CPPU standard, washed with 2 mL of water and eluted using 2 mL of 2-propanol/water from 0 to 100% (v/v) in independent experiments. CPPU began to be detected in the eluted fractions at solvent concentrations above 20%. Accordingly, 2-propanol at 10% (v/v) in water was selected as washing solution, while pure 2-propanol was employed for analyte elution because it provided satisfactory recoveries (from 100 to 105%) in a 2 mL fraction.

The analytical effectiveness of the developed IAC-IMS procedure for residue analysis was evaluated using water and kiwi juice spiked with CPPU at three concentration (10, 20, 40 and 400 µg L<sup>-1</sup>) levels. Different sample volumes were loaded on the IAC depending on the spiked level, and recoveries were determined by IMS, obtaining in all the cases quantitative recoveries.

**Acknowledgements:** Authors gratefully acknowledge the financial support of the Ministerio de Economía y Competitividad (CTQ 2014-52841-P) and Generalitat Valenciana (project PROMETEO-II 2014-077).

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# MOLECULARLY IMPRINTED POLYMERS AS SAMPLE TREATMENT FOR THE DETERMINATION OF IMIDACLOPRID IN SOIL AND PLANTS BY ION MOBILITY SPECTROMETRY

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Ion mobility spectrometry (IMS) is a gas phase ion separation technique at ambient pressure, in which ions are separated according to their individual velocities as they drift through an inert gas driven by an electric field. For a typical IMS device operating under normal conditions, the measured number of theoretical plates rarely exceeded 5000. Thus, it is easy to imagine that the lower separation efficiency has limited the applicability of the technique. Therefore, the incomplete resolution of peaks is a common situation in the analysis of complex samples by IMS. In recent years, the selectivity of sample treatments have been improved by using analyte-selective supports, such as immunoaffinity chromatography columns [1] and molecularly imprinted polymers (MIPs) [2]. The main advantage of MIPs in front of the other selective supports are related to their chemical inertness, long-term stability, possibility to synthesize large quantities, high sorption capacity, and relatively low acquisition cost.

The aim of this study was the development of MIP for the determination of imidacloprid in soil and plants by IMS. Imidacloprid (1-(6-chloro-3-pyridinylmethyl)-N-nitroimidazolidin-2-ylideneamine) is a systemic, chloro-neonicotinyl insecticide that blocks nicotinergeric neuronal pathways.

The procedure is based on the extraction of imidacloprid from soil and plants using 50 mL hot water, its preconcentration by MIP and later analysis by IMS. The method was validated in terms of precision, limit of detection (LOD) and quantification (LOQ) and recovery of imidacloprid in spiked matrices. The relative standard deviation (RSD) was lower than 5% for three analysis at different concentration levels (0.4, 1 and 2  $\mu\text{g g}^{-1}$ ). The LOD and LOQ values of the procedure were 0.05 and 0.15  $\mu\text{g g}^{-1}$ , respectively. Recoveries of imidacloprid from water, soil and tomato plants were in all the cases higher than 90%.

Contaminated soils at 50  $\text{mg kg}^{-1}$  concentration level were used for this study. 2 L of acetone containing the corresponding amount of imidacloprid standard was added to 25 kg of soil and mixed thoroughly. After air drying for 48 h, the soils were mixed to ensure homogeneity and prior to growing tomato and chili plants, the imidacloprid concentration in soil was measured.

Small tomato and chili seedlings (approximately 10 cm height and 15–20 g) were transplanted into individual pots with 750 and 350 g of imidacloprid spiked soil, respectively. The plants were grown over 20, 30 and 60 days under natural light, being the air temperature and humidity monitored over the growing duration. The water retention capacity of the soil was experimentally determined to be a minimum of 10 mL, therefore each plant was watered daily with 10 mL of water. No excess water resulted from this process. Imidacloprid determination was carried out on the soil and tomato and chili plants (roots, stem and leaves).

**Acknowledgements:** The authors gratefully acknowledge the financial support of the Ministerio de Economía y Competitividad (Project CTQ 2014-52841-P) and Generalitat Valenciana (project PROMETEO-II 2014-077).

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## SELENIUM SPECIES DETERMINATION IN AMAZON FOODS BY HPLC – ICP-MS AFTER ENZYMATIC HYDROLYSIS ASSISTED BY PRESSURISATION AND MICROWAVE ENERGY

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Selenium is a minor and essential dietary element for human, which is involved in antioxidant defense, thyroid homeostasis and regulation of redox status; preventing cancer risk and thyroid disorders; and protect neurons, skeletal and cardiac muscles [1]. Organoselenium compounds (such as selenoaminoacids) are of major biological relevance in mammals due to their role in active centers of selenoenzymes (selenoprotein P and selenogluthathione peroxidase) [1,2]. The main source of selenium for human is the diet. 55 µg Se/day is generally the recommended intake of selenium amount (NAH, 2000). However, the content of selenium in the soil, and thus in the crops grown in them, of several regions of the world (is insufficient to offer the proper protective activity [1,3]. This fact justifies the growing interest in the production of selenium fortified foods and selenium-based nutritional supplements. Exotic nuts and fruits from Amazon region such as Brazil nut, golden berries and heart of palm are considered as functional foods or “super foods” due to their nutritional value (high levels of antioxidants, essential fatty acids, vitamins, essential amino acids, and essential minerals such as Se [4]).

Hyphenated analytical techniques based on the coupling of chromatographic separation ICP-MS are the basic tools to identify and quantify selenium species in biological samples. Mass spectrometers having a softer ionization source than ICP are also utilized for the identification of unknown selenocompounds [1]. Sample preparation is a crucial step for Se speciation in food. Specific sample pre-treatment procedures are based on enzymatic extraction, protein precipitation, ultrafiltration and sonication have been recently proposed.

In this communication, total selenium and selenium species extraction from different raw Amazon foods (Brazil nut, Inca golden berries and heart of palm) has been assessed by using enzymatic hydrolysis approaches assisted or accelerated by pressurization or microwave energy. Selenium species was released from dried and defatted foods by the action of a protease (protease XIV) and an enzyme activator (DTT, dithiothreitol). ICP–MS was used to assess total selenium contents after a microwave assisted acid digestion, and also to quantify total selenium in the enzymatic extracts. Selenium speciation in the enzymatic extracts was assessed by high performance liquid chromatography (HPLC) coupled with ICP-MS detection. Major Se species (Selenocystine SeCys<sub>2</sub> and Selenium methionine SeMet) from enzymatic extracts were identified and characterized by HPLC coupled to mass spectrometry (HPLC–MS). Several variables inherent to the enzymatic activity (TRIS-HCl concentration, TRIS-HCl volume, pH, temperature, and protease XIV and DTT masses) and variables affecting pressurization and / or microwave extraction (pressurized time, pressure and microwave extraction steps) were studied. Analytical performances, such as limits of detection and quantification, repeatability and accuracy of the over-all procedures were established. SeMet were found at high concentration in the enzymatic extracts from all samples. SeCys<sub>2</sub> and Se-(Methyl)selenocysteine (SeMeCys) were detected at low concentrations in some samples while oxidized selenium methionine (SeOMet) and inorganic selenium species (selenite and selenate) were not detected in the enzymatic extracts from Amazon samples.

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### Acknowledgement

This research was supported by the Galician Competitive Research Group GRC2013-047. We are grateful to Alicia María Cantarero-Roldán (Servicios Xerais de Apoio a Investigación at the University of A Coruña) for ICP-MS technical support.

## HARD CAP ESPRESSO EXTRACTION OF CANNABINOIDS FROM CANNABIS

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Hard cap espresso machine has been recently employed for the extraction of several organic compounds from relevant solid samples. In this approach, samples are introduced in refillable stainless steel capsules and target analytes are extracted in few seconds using acetonitrile:water or ethanol:water mixtures at a fixed and moderate temperature and pressure conditions of 72 °C and 19 bar, respectively. The use of hard cap espresso machines provided an easy, fast, low-cost and reliable extraction method with multiple potential applications, such as: polycyclic aromatic hydrocarbons from soils [1], polychlorinated biphenyls from soils [2], bioactive compounds from spices and herbs [3], and pesticides from airborne PM10 filters [4].

In this study, a hard cap espresso machine has been used for the quantitative extraction of cannabinoids from cannabis flowers and leaves. The proposed hard cap espresso extraction allows a quantitative, simple and rapid ( $39 \pm 2$  s) extraction of cannabinoids from cannabis plants.  $\Delta^9$ -tetrahydrocannabinol (THC), cannabinol (CBN) and cannabidiol (CBD) were the major cannabinoids identified in the extracts by gas chromatography-mass spectrometry (GC-MS) with characteristic mass fragments of 231, 246 and 174 m/z for CBD; 299, 314 and 231 m/z for THC; and 295, 238 and 310 m/z for CBN.

Linearity of the method was established using calibration curves of THC dissolved in 2-propanol with triphenyl phosphate  $1 \mu\text{g mL}^{-1}$  as internal standard. Satisfactory coefficients of determination ( $R^2$ ) ranging from 0.994 to 0.999 were found. Precision was established as the relative standard deviation (RSD) of the lowest concentration THC standard ( $0.05 \mu\text{g mL}^{-1}$ ), with a value of 14%. Limits of detection (LOD) and quantification (LOQ) were calculated as 3 and 10 times, respectively, the standard deviation of the intercept of the calibration line divided by the calibration slope. LOD and LOQ values of 0.2 and  $0.7 \mu\text{g mL}^{-1}$  were obtained.

The effect of solvent nature (pure acetonitrile and 2-propanol) and volume (consecutive extractions of 50 mL) of the extraction solution were evaluated to obtain quantitative recoveries. Thus, 0.2 g of cannabis were extracted with 50 mL 2-propanol and the extracted cannabinoids were directly determined by GC-MS.

The extraction efficacy of the proposed method, using a hard cap espresso machine, was also evaluated by method comparison using ultrasound assisted (UAE) reference procedure for the analysis of real samples. The analyzed cannabis samples (flowers and leaves) were also extracted by UAE and analysed by GC-MS with THC concentrations ranging from 16 to 28 and 0.71 to  $0.81 \text{ mg g}^{-1}$ , respectively. The proposed hard cap espresso extraction provides results statistically comparable to those obtained using the reference UAE procedure, being an effective, low cost and rapid extraction methodology.

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**DERIVATIZACIÓN *IN SITU* COMBINADA CON MEPS PARA LA DETERMINACIÓN DE CLOROFENOLES EN SUELOS POR CROMATOGRAFÍA DE GASES-ESPECTROMETRÍA DE MASAS**

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Se propone un método analítico basado en la derivatización y extracción mediante microextracción con sorbentes empaquetados (MEPS) para la determinación de clorofenoles en suelos. Las especiales características de MEPS permiten su acoplamiento con cromatografía de gases (GC) facilitando la automatización del procedimiento.

La derivatización de los clorofenoles se llevó a cabo mediante una reacción de acilación en medio básico, ya que ésta puede realizarse *in situ* en medio acuoso, es rápida (unos pocos minutos), presenta elevados rendimientos y bajo coste en reactivos [1]. Además, la fase líquida extraída resulta adecuada para la preconcentración de los extractos mediante MEPS. En el procedimiento se usa un vaporizador de temperatura programado (PTV) que funciona en modo de purga de disolvente para inyectar los extractos en el cromatógrafo de gases consiguiendo un efecto adicional de preconcentración de los analitos.

Se estudiaron las diferentes variables que afectan al proceso de derivatización y al de extracción mediante MEPS y se comprobó, en las condiciones experimentales optimizadas, la existencia de efecto de matriz debido a la complejidad de las matrices estudiadas. Se propuso, por tanto, el método de adiciones estándar para la determinación de los analitos en las muestras.

Se realizaron los calibrados de todos los compuestos en la matriz del suelo y todos ellos mostraron un comportamiento lineal sin fallo de ajuste, con buenos valores de repetitividad y reproducibilidad (RSD inferiores al 10 %). Los límites de detección del procedimiento propuesto estaban en el margen comprendido entre 0.118–0.894 g kg<sup>-1</sup> de suelo.

La validación del método propuesto se llevó a cabo determinando la concentración de los clorofenoles en un material de referencia certificado (CRM136). Los resultados obtenidos ponen de manifiesto la validez del método propuesto.

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## HS-GC-MS PARA LA DETERMINACIÓN RÁPIDA DE POSIBLES BIOMARCADORES EN MUESTRAS DE ORINA

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El desarrollo de estrategias analíticas rápidas juega un papel importante en la investigación, descubrimiento y confirmación de analitos que pueden ser utilizados como posibles biomarcadores para diferentes tipos de enfermedades. En este contexto, se ha propuesto y validado un método analítico rápido basado en el acoplamiento un generador de espacio de cabeza con cromatografía de gases y espectrometría de masas (HS-GC-MS) para la determinación de posibles biomarcadores (disulfuro de dimetilo, tolueno, 1,4-xileno, 2-octanona y 2,6-dimetil-7-octen-2-ol) en muestras de orina.

La utilización de generación de espacio de cabeza presenta las ventajas de que el tratamiento de la muestra es mínimo y evita la presencia de interferencias de compuestos no volátiles presentes en la matriz, lo que hace de esta técnica una excelente opción para matrices complejas como son las muestras de orina. En el trabajo se estudiaron las variables que afectan al espacio de cabeza (pH y volumen de muestra y temperatura y tiempo de equilibrio), fijándose como valores adecuados pH fisiológico, 3 mL, 90 °C y 30 min, respectivamente. La rapidez del análisis se consigue utilizando el modo de inyección de *split* en caliente e inyectando directamente los volátiles generados sin someterlos a ningún proceso de preconcentración adicional.

En las condiciones experimentales optimizadas se comprobó la existencia de efecto de matriz por lo que para la cuantificación se propuso un método de adición estándar de un punto. Se determinaron las rectas de calibrado y se comprobó que no existía fallo de ajuste en ningún caso. El procedimiento propuesto presentó valores adecuados de repetitividad y reproducibilidad (RSD inferior a 9.5 % y 14.2 %, respectivamente) y unos límites de detección entre 0.01 y 0.48 mg L<sup>-1</sup>.

Para la validación del método propuesto se utilizaron muestras de orina de pacientes sanos dopadas con los analitos estudiados. Los resultados obtenidos demuestran que el método propuesto resulta adecuado para la determinación de estos compuestos en estas matrices.

**DETERMINATION OF RESIDUES OF FLUOROQUINOLONES IN CHICKEN MUSCLE BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY WITH DAD DETECTION USING A MODIFIED QUECHERS METHOD****H. Ponce Rodríguez\*, N. Lanza Sorto, A. Blanco Raudales**

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Fluoroquinolones are antimicrobial agents widely used in veterinary practice to treat diseases, to control and prevent infection and to promote growth and production efficiency [1]. However, it is necessary that these compounds antibiotics are within the limits as insurance at the time of being consumed by humans, so that they do not represent a risk to health due to antibiotic resistance. In that sense, some international standards already established residue levels of these compounds (MRL), like the Council regulation of the European Union EEC 2377/90 and Codex Alimentarius of FAO. In Honduras, these limits are adopted which established the codex Alimentarius [2], with accepted values for Danofloxacin, Flumequine and Sarafloxacin of 200 µg/Kg, 500 µg/Kg, and 10 µg/Kg respectively in chicken muscle.

To date, many have been works and scientific publications on the development of analytical methods to determine these compounds in food matrix [3], using many techniques such as liquid chromatography, capillary electrophoresis and gas chromatography. In the case of the preparation of the sample most of these publications concern the use of the extraction phase solid (SPE) as a technique of extraction and concentration prior to the chromatographic analysis. However, the QuEChERS, Introduced by Anastassiades and collaborators in 2003 [4], application for the study of these substances it has gained popularity due to the advantages it presents.

A method is proposed using a Accucore C18 (100 x 4.6 mm x 2.6 µm) partially porous column of Thermo, the mobile phase consisted of (A): 0,1M acetic acid aqueous solution (pH 2.5) and (B): Methanol. A linear gradient was selected for the separation with the following program: 0 min 15% B; 10 min 15% B; 15 min 20% B; 20 min 100% B. Analysis was performed at a flow rate of 1mL/min, with an injection volume of 20µL and two wavelengths, 250 nm for flumequine and 280nm for the rest of compounds, including Ciprofloxacin, Danofloxacin, Difloxacin, Sarafloxacin and Norfloxacin (SI). Different parameters of the QuEChERS method was optimized, including volume of extractor solvent, type of salts, centrifugation time, use of SPE dispersive step to obtain high recoveries percentages.

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## EXTRACTION OF ARSENIC SPECIES FROM SEAFOOD SAMPLES BY USING A HARD CAP ESPRESSO MACHINE

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A Nespresso hard cap espresso machine has been employed for the quantitative extraction of arsenic from seafood. Sample extraction was performed from 0.5 g in less than 40 s, with 50 mL of different acid solutions such as citric acid (5% m/v), nitric acid (0.1M), H<sub>2</sub>SO<sub>4</sub> (0.1 M), HCl (0.1 M), acetic acid (0.1 M) and H<sub>3</sub>PO<sub>4</sub> (0.1M) at a temperature of 72 ± 3°C and a pressure of 19 bar.

Toxic arsenic was determined by hydride generation-atomic fluorescence spectrometry (HG-AFS). To evaluate the accuracy and robustness of the extraction method, a value of toxic As was obtained by a reference method, which consisted on microwave-assisted extraction. Obtained concentration of total arsenic extracted by Nespresso coffee machine was 480 ± 60 ng/g for clam samples with phosphoric acid. This value was much closed to the concentration obtained with the reference method which was 440 ± 60 ng/g.

For swordfish samples, studies are in due course to evaluate the capability of the extraction method to determine arsenic. Inorganic As in both, clam and swordfish, were also under study.

The figure shows the amount of As extracted in different portions of 50 mL H<sub>3</sub>PO<sub>4</sub> 0.1 M.

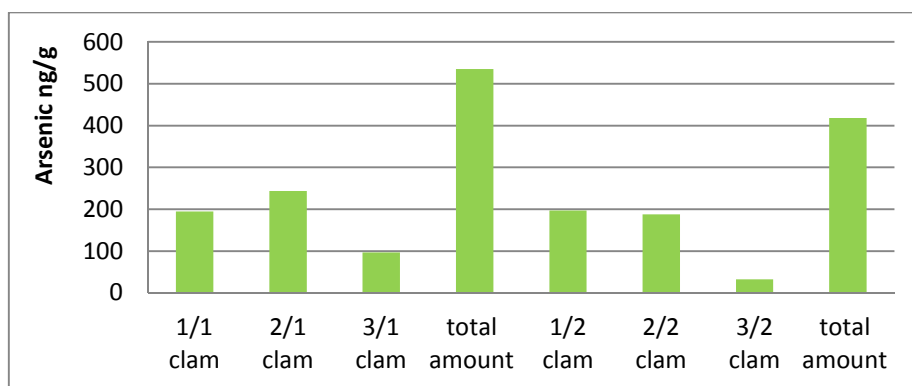


Figure. Amount of As extracted by Nespresso from clam with 0.1 M H<sub>3</sub>PO<sub>4</sub>

Acknowledgement: Authors acknowledge the financial support of Generalitat Valenciana Project PROMETEO II 2014-077.

**DISPOSITIVO MICROFLUÍDICO ON-CHIP PARA LA EXTRACCIÓN SIMULTÁNEA DE  
ANTIINFLAMATORIOS NO ESTEROIDEOS Y PARABENES MEDIANTE  
MICROEXTRACCIÓN EN FASE LÍQUIDA Y SU APLICACIÓN EN AGUAS SUPERFICIALES**

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En este trabajo se pone a punto un procedimiento de microextracción en fase líquida integrada en un sistema microfluídico on-chip, para la extracción simultánea de dos familias de compuestos: cinco antiinflamatorios no esteroideos y cuatro parabenos.

El dispositivo microfluídico construido con polimetil-metacrilato consistió en dos micro-canales (aceptor y donador) separados por una membrana plana de polipropileno de 25  $\mu\text{m}$  de espesor. Los nueve analitos de estudio son extraídos desde la fase donadora hasta la fase aceptora a través de la membrana gracias a un gradiente de pH entre ambas disoluciones acuosas. El pH de la fase donadora y aceptora fue de 3.5 y 12, respectivamente. Las fases donadora /muestra y aceptora se introdujeron en el dispositivo microfluídico impulsado por dos bombas de jeringa. Para la optimización se probaron flujos en el rango de 1–30  $\mu\text{L min}^{-1}$  y de 1–4  $\mu\text{L min}^{-1}$  para la fase donadora y aceptora, respectivamente. Paralelamente, el dispositivo fue probado en condiciones de *stopped-flow*. Finalmente, el extracto extraído se analizó directamente mediante HPLC.

Las recuperaciones obtenidas en muestras reales fueron superiores al 85 % en todos los casos, obteniendo excelentes líneas bases. Además, las extracciones se completaron en menos de seis minutos y con volúmenes de muestra requeridos inferiores a los 200  $\mu\text{L}$ .

**Agradecimientos:** BP B-Marie Curie (0025) (the 7th Framework Programme European Commission) y el Ministerio de Economía, industria y competitividad- contrato Juan de la Cierva Incorporación.

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**USE OF MULTIPLE HEADSPACE SAMPLE ENRICHMENT (MHSE) TO IMPROVE SENSITIVITY IN HEADSPACE GAS CHROMATOGRAPHY**

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The use of headspace sampling (HS) coupled to gas chromatography (GC) solves many analytical problems by minimizing sample treatment. However, in many cases the limits of detection achieved are insufficient for the detection or quantification of the analytes of interest, and additional preconcentration steps are required. Among the techniques used for this purpose solid-phase microextraction (SPME) and headspace single-drop microextraction (HS-SDME) can be cited.

The use of a programmed temperature vaporizer (PTV) inlet offers another alternative for increasing sensitivity. When using the solvent vent injection mode, the analytes are focused cryogenically in the liner of the injector, whilst major compounds that are more volatile, are eliminated. Later application of a rapid temperature ramp allows these analytes to be introduced into the GC column, with the advantage that a considerable narrowing of the chromatographic peaks occurs.

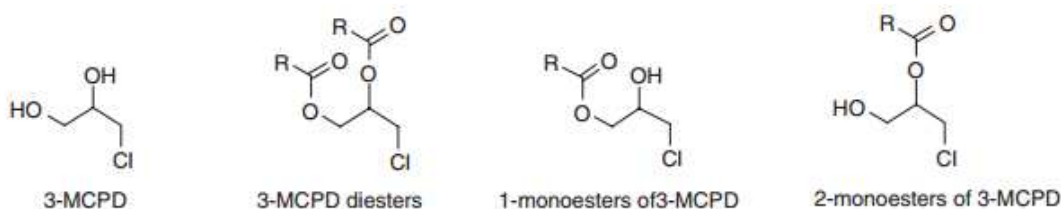
In this work, other possible use of the PTV, coupled with HS, is proposed: multiple headspace sample enrichment (MHSE). This option allows multiple injections from the same vial into the cold inlet for enhanced analyte detection. The vial is pressurized with helium between injections, and the volatiles are repeatedly extracted from the sample matrix. The liner of the PTV is used as a cold trap, and the split valve is open to eliminate the excess gas, until injection into the GC column gas chromatograph. Different materials in the liner (glasswool, Tenax-TA, Carbotrap) can be used as an additional factor for modifying the selectivity of the process. This approach has been applied to different volatile compounds with promising results.

## DETERMINACIÓN SIMULTÁNEA DE ÉSTERES DE ÁCIDOS GRASOS DE 3-MCPD EN ACEITES DE USO ALIMENTARIO MEDIANTE GC-MS UTILIZANDO UN PROCESO DE HIDRÓLISIS ALCALINA

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En el punto de mira de la calidad alimentaria están los ésteres de 3-monocloropropano-1,2-diol (3-MCPD) con diferentes ácidos grasos, considerados como potencialmente cancerígenos, de los cuales según la legislación [1] se establece un consumo máximo de  $0.8 \mu\text{g/kg}$  de persona al día. Estos compuestos pueden estar presentes en los alimentos procesados puesto que se forman en los aceites de uso alimentario cuando se refinan a altas temperaturas ( $> 200^\circ\text{C}$ ).



**Figura 2.-** 3MCPD y sus diferentes mono y diésteres

Los ésteres de 3-MCPD con ácidos grasos se analizan por cromatografía de líquidos (LC) de manera directa puesto que son compuestos no volátiles. Su determinación por LC plantea problemas desde el punto de vista de la sensibilidad y de la elevada cantidad de estándares requerida para cuantificar cada uno de ellos de forma individual.

En este trabajo se plantea como alternativa, la hidrólisis de los enlaces éster (puede realizarse en medio ácido o básico, o incluso por acción enzimática) para liberar 3-MCPD [2], y una posterior microextracción líquido-líquido dispersiva, cuantificando el 3-MCPD liberado por cromatografía de gases-espectrometría de masas (GC-MS). La hidrólisis se ha llevado a cabo en medio básico con metóxido sódico con salting-out y se combina con un proceso de concentración y purificación mediante DLLME [3] que incluye la derivatización con HFBI. El método fue validado en términos de linealidad, límites de detección (LOD) y cuantificación (LOQ), precisión y exactitud. Se obtuvo un rango lineal entre  $5 - 500 \text{ ng mL}^{-1}$  para 3-MCPD en su forma libre con coeficientes de determinación de 0.998. Se alcanzaron límites de detección y cuantificación de  $2 \text{ ng mL}^{-1}$  y  $5 \text{ ng mL}^{-1}$  respectivamente. La precisión fue inferior al 7% RSD y la recuperación próxima al 100%.

### Acknowledgements

Los autores agradecen la financiación de este trabajo al Proyecto AGL2014-53647-R Plan Estatal de Investigación Científica y Técnica y de Innovación 2013–2016 and FEDER y al Proyecto SPIP2015-01838 de la DGT Ministerio del Interior (España).

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## BORONATE FUNCTIONALIZED POLYMER MONOLITHS AS SPE SORBENTS AND ITS APPLICATION FOR ENRICHMENT OF GLYCOPROTEINS

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Glycoproteins contain oligosaccharides currently attached to the polypeptide side chains. The identification of glycoproteins played an important role in diseases diagnoses and treatments. The selective isolation and enrichment of glycoproteins from complex biological samples constitutes indispensable step in almost all branches of biosciences and biotechnologies, but they remain challenging tasks due to their low abundance. Recently, the fabrication of novel polymer monoliths modified with gold nanoparticles (AuNPs), offering high permeability and surface area, are effective and reliable SPE sorbents for protein extraction has received much attention [1, 2].

In addition, these AuNP-modified materials can be considered as promising “tuneable” support for immobilizing boronic acid groups, which can react with the adjacent diol groups, commonly present in the glycoproteins. With regard to SPE devices, pipette tips are considered as a potential alternative to the traditional SPE cartridges, since they reduce the amount of solvents and sample volume, thus reducing the processing time. Concretely, the use of porous monoliths as sorbents for sample preparation in pipette tip technology has been described [3, 4].

In this work, a porous polymer monoliths modified with AuNPs placed into a pipette tip for isolation of proteins has been developed. Firstly, monolith methacrylate-based monoliths prepared in 200 µL pipette tips were modified with cystamine to provide thiol groups onto the pore surface of the material for the subsequent attachment of AuNPs. Then, a subsequent functionalization of these AuNPs by reaction with boronic acid derivatives was accomplished. The analytical features of SPE sorbent were optimized using ovalbumin as test solute. The applicability of this sorbent was demonstrated by isolating glycoproteins from another non-glycosylated protein as BSA, followed by SDS-PAGE analysis.

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**Acknowledgements:** Projects CTQ2014-52765-R (MINECO of Spain and FEDER) and GV-PROMETEO2016-145 (Conselleria de Educació, Investigació, Cultura y Deporte, Generalitat Valenciana, Spain). M. V-B thanks the MEC for an FPU grant for PhD studies.

## HARD CAP ESPRESSO MACHINE EXTRACTION OF POLYPHENOLIC COMPOUNDS FROM FOOD

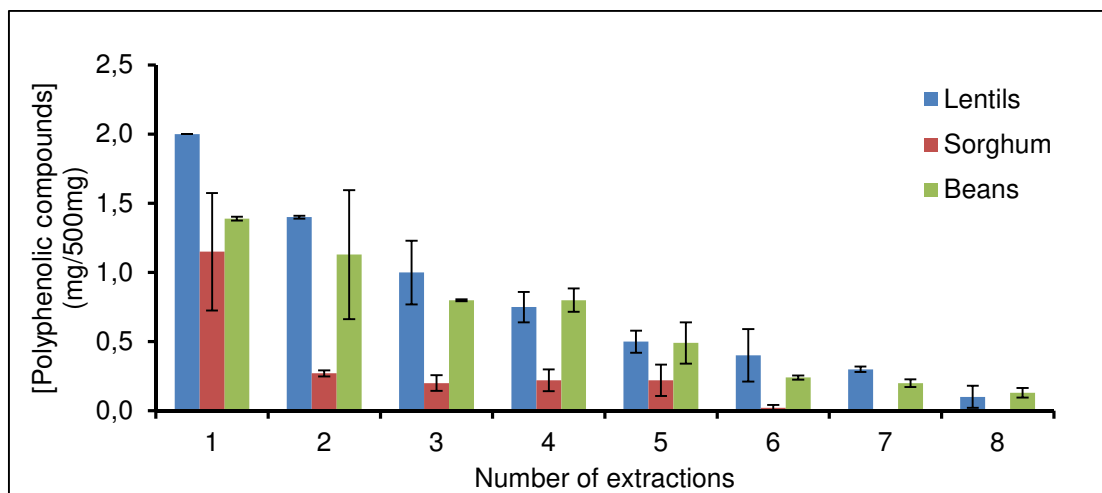
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A hard cap espresso machine was evaluated for the extraction of polyphenolic compounds from food samples as lentils, beans and sorghum. 500 mg of dry sample were diluted with Spe-ed<sup>TM</sup> dispersing agent inside an 8.8 mL stainless steel capsule which was loaded into the machine. The extraction of polyphenols was evaluated by the use of successive 50 mL fractions of different solvents like water, ethanol 50% (v/v) in water and ethanol 80% (v/v) in water. The obtained extracts were analyzed spectrophotometrically by the use of the Folin-Ciocalteu method. Extraction volumes of 350, 300 and 250 mL ethanol 50% (v/v) in water, involving times from 65 to 90 s, were enough to provide a quantitative extraction of lentils, beans and sorghum, respectively, as compared with a reference method based on the use of ultrasound-assisted extraction (see results in Figure 1).

The obtained levels of polyphenolic compounds were 12.22, 9.72 and 4.34 mg/g for lentils, beans and sorghum, respectively. In short, the developed procedure provides a fast extraction at mild conditions of 72°C and 19 bars of phenolic compounds present in foods.



**Table 1.** Total polyphenol content in lentils, beans and sorghum extracted by hard cap espresso extraction using successive volumes of 50 mL ethanol 50% (v/v) in water.

Acknowledgements: Authors gratefully acknowledge the financial support of the Ministerio de Economía y competitividad (CTQ 2014-52841-P) and Generalitat Valenciana (project PROMETEO-II 2014-077).

## STUDY OF THE EFFICIENCY OF CHROMATOGRAPHIC COLUMNS IN GRADIENT ELUTION

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The study of column performance in liquid chromatography is of great interest to characterise stationary phases, develop new materials and increase the resolution of complex samples. Traditionally, column performance in liquid chromatography has been studied using information from the isocratic elution of probe compounds at different flow rates through van Deemter plots, which relate the column plate height to the linear mobile phase velocity. Dispersion of chromatographic peaks depends on the processes that take place inside the column along solute elution, such as eddy diffusion, molecular longitudinal and axial diffusion, convection, multiple elution paths, and slow mass transfer between mobile phase and stationary phase.

A procedure is proposed to study the dispersion parameters as a function of the flow rate, using linear gradients. The effects of the flow rate on peak broadening in gradient elution and the different mechanisms that predominate at low and high values of the flow rate are examined. Models to predict the peak half-widths and variance are proposed in gradient elution. For the right half-width, the next equation describes the behaviour:

$$A_g = r \times \frac{1 - e^{-S m t_0 k_g}}{S m} + A_0$$

where  $A_g$  is the right half-width,  $t_0$  the dead time,  $A_0$  the right half-width of a peak eluted at dead time,  $k_g$  the retention factor of the compound eluted in a linear gradient with an  $m$  gradient rate, and  $S$  the elution strength of the compound.

The results are compared with those obtained in isocratic elution to establish the effect of peak compression in gradient elution, and predict the best separation conditions. The study was carried out with four sulphonamides, separated with two reverse-phase columns of different nature (Zorbax and Chromolith SpeedROD), using acetonitrile-water mixtures.

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# EXTENT OF THE INFLUENCE OF PHOSPHATE BUFFER AND IONIC LIQUIDS ON THE REDUCTION OF THE SILANOL EFFECT IN A C18 STATIONARY PHASE

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The presence of anionic free silanols in the silica-based stationary phases gives rise to broad and asymmetrical peaks when cationic basic compounds are chromatographed using hydro-organic mobile phases. The addition to the mobile phase of a reagent with ionic character prevents the access of analytes to the free silanols, improving the peak shape. The silanol activity can be affected by the buffer concentration and mobile phase pH, factors that are not always considered sufficiently in the literature. In this work, the chromatographic behaviour of three basic  $\beta$ -adrenoceptor antagonists (acebutolol, nadolol and timolol), using mobile phases containing acetonitrile, was examined at different phosphate buffer concentrations (5 to 50 mM) and mobile phase pH (2 to 8), in the absence and presence of three imidazolium-based ionic liquids (1 ethyl-, 1 butyl- and 1-hexyl-3-methylimidazolium chloride). All factors were evaluated through both the retention and peak shape. The imidazolium cations can block the access of cationic analytes through electrostatic interaction with the anionic silanols, or association with the alkyl chains bound to the stationary phase. In previous reports, the protection mechanism was demonstrated to be directly related to the cation size. The studies in this work reveal that the effectiveness of the mobile phase additive as silanol blocker also depends on the concentration of the buffer anion and the amount of non-protonated silanols on the stationary phase. Increasing amounts of phosphate at low pH give rise to increasing retention times. Also, the peak shape is improved, which indicates the influence of phosphate on blocking the activity of free silanols. However, the benefits obtained by the combined effect of buffering the mobile phase at low pH and the use of a bulky additive are lost at pH > 6.

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**MICROEMULSION LIQUID CHROMATOGRAPHY:  
A USEFUL TECHNIQUE FOR DRUG ANALYSIS****S. Carda-Broch<sup>1</sup>, E. Peris-García<sup>2</sup>, M.J. Ruiz-Angel<sup>2</sup>, M.C. García-Alvarez-Coque<sup>2</sup>**<sup>1</sup>Department of Physical and Analytical Chemistry, University Jaume I,  
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Microemulsions are stable, isotropically clear solutions consisting of two immiscible liquids (e.g. water and an oil, such as octane) stabilized by a surfactant, which can be mixed with a co-surfactant. The interfacial tension between the oil and water has to be decreased to yield the mixture. Sodium dodecyl sulphate is a common and widely used surfactant, whereas short chain alcohols, such as butanol or pentanol, are usual co-surfactants. Microemulsions contain nanometre-sized surfactant coated droplets of oil suspended in water, which are called oil-in-water (o/w) microemulsions. The use of microemulsion mobile phases with conventional reversed-phase columns is known as microemulsion liquid chromatography (MELC), which is considered an extension of micellar liquid chromatography. Microemulsions have a high solubilizing power, give rise to a particular selectivity, and show interesting features for gradient elution. A thorough revision of the published MELC procedures for the determination of drugs in clinical and pharmaceutical samples is presented, which includes the nature of surfactant, oil, co-surfactant, and the column type. The objective was to study the trends in the use of MELC in drug analysis.

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## DESARROLLO DE UN MÉTODO CROMATOGRÁFICO PARA LA DETERMINACIÓN DE AMINAS BIÓGENAS EN CERVEZA

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Las aminas biógenas (ABs) son compuestos que aparecen de forma natural en el organismo, y que participan en procesos fundamentales como la síntesis de ácidos nucleicos y proteínas [1]. Sin embargo, la producción endógena de las mismas es insignificante comparada con el consumo exógeno, ya que aparecen en multitud de alimentos<sup>2</sup> (siendo las bebidas fermentadas como la cerveza uno de ellos).

Su ingesta innecesaria puede causar alteraciones en la salud, desde dolor de cabeza, rubor, picazón, desórdenes en la respiración, vómitos, alteraciones en la presión arterial, palpitaciones y taquicardia, hasta choques anafilácticos en sujetos sensibles. Niveles elevados en el cerebro se han asociado a la enfermedad de Alzheimer, Parkinson y esclerosis lateral amiotrófica. El grupo amino puede reaccionar también con los nitritos y generar compuestos cancerígenos. Se consideran además marcadores tumorales [2-5].

En los últimos tiempos, debido al gran consumo mundial de cerveza y el aumento de producción artesana, ha cobrado especial relevancia el control de las ABs durante su producción, ya que los tipos y niveles de ABs dependen tanto de los materiales de partida como del proceso de fabricación, así como de posibles contaminaciones microbianas [6]. Por ello, los niveles de ABs pueden emplearse como índice de calidad del proceso de fabricación de la cerveza[7].

En la presente investigación se está optimizando un método de UHPLC-FLD para la determinación de ABs (etanolamina, agmatina, putrescina, cadaverina, histamina, tiramina, triptamina y feniletilamina), previa derivatización con OPA. Se ha empleado un diseño de experimentos para la optimización de la composición de la fase móvil, siendo ésta finalmente la formada por tampón TRIS (0,08 M, pH 8,8; 3,5% THF), MeOH y ACN. La separación de las ABs se consigue en 15 minutos mediante elución en gradiente.

La intensidad de fluorescencia de los productos derivatizados se ve altamente influenciada por el pH. De esta manera, al pH óptimo de la separación (básico), se observa una disminución de la señal de fluorescencia. Por tanto, se está intentando modificar el pH de la fase móvil tras la separación en la columna, para aumentar así la señal de fluorescencia.

Una vez optimizado el método, se estudiará la relación lineal entre área o altura de pico y concentración, para proceder posteriormente a la identificación y determinación de las ABs durante el proceso de producción de cerveza.

**Agradecimientos.** Los autores agradecen la financiación de este trabajo al Ministerio de Economía y Competitividad de España (Proyecto CTQ2014-52309-P) y a la Junta de Extremadura (IB16058 y GR15090-Grupo de investigación FQM003). M. Palomino-Vasco agradece a la Junta de Extremadura la concesión de su contrato predoctoral (PD16033). Todos los proyectos están cofinanciados por los Fondos Europeos FEDER.

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## CAPILLARY LC OF METALLIC NANOPARTICLES COUPLED ON LINE TO IT-SPME: APPLICATION TO PLASMONIC ASSAYS.

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In-tube solid-phase microextraction coupled to capillary liquid chromatography with diode array detection (IT-SPME Cap-LC-DAD) is a new tool for estimating mean concentrations of Ag/AuNPs and thereby determine their average size [1]. Size-exclusion and hydrophobic effects are the mechanisms involved to explain the chromatographic profiles of the nanoparticles. The proposed technique allows the direct analysis of AuNPs in different matrices, providing new information for their characterization. In addition, it has been studied the effect of solvent on the dispersions stability and their aggregation.

In this work, the possibilities of IT-SPME Cap-LC-DAD to monitor plasmonic assays have been studied. As an example, the spermine colorimetric assay previously developed by this research group is addressed [2]. The chromatographic profiles showed significant differences in the aggregation state in function of the spermine concentration, mainly in the chromatographic peak corresponding to hydrophobic interaction mechanism. Urine samples from cancer patients and healthy volunteers were analysed by the proposed method. The assay discriminates between cancer patients and healthy volunteers responses (see Figure 1). Therefore, IT-SPME Cap-LC-DAD is a potential tool to monitor NPs plasmonic assays in clinical analysis.

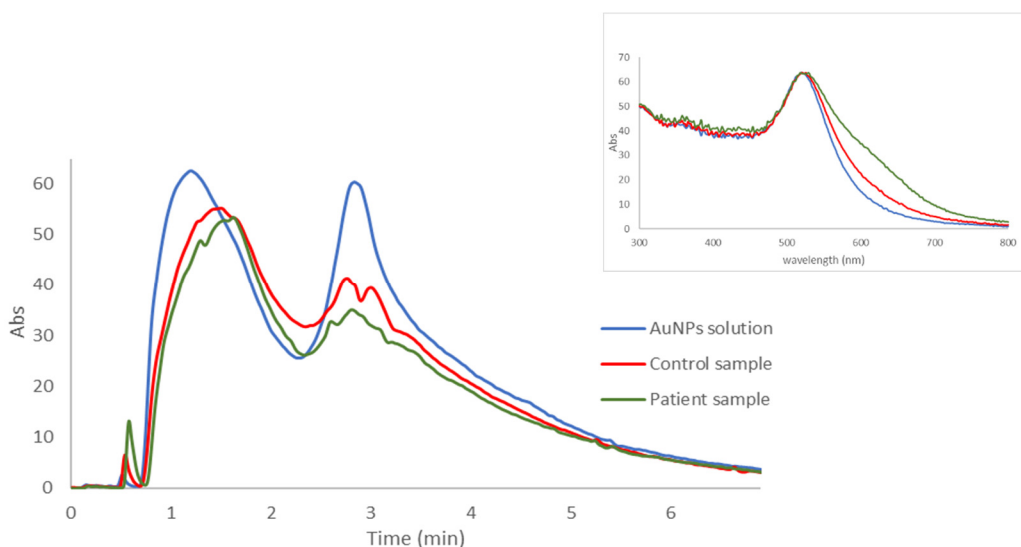


Figure 1. Chromatographic profiles for AuNPs solution (blue), control sample from healthy volunteers (red) and cancer patient sample (green). Inset: UV-Vis spectra of the chromatographic peak at 2.90 min.

### Acknowledgement

The authors are grateful to Generalitat Valenciana (PROMETEO 2016/109) and to the Spanish Ministerio de Economía y Competitividad (MINECO) and EU-FEDER (project CTQ2014-53916-P) for financial support.

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**PREPARATION AND CHARACTERIZATION OF HYBRID CAPILLARY MONOLITHIC COLUMNS WITH MESOPOROUS SILICA PARTICLES FOR SEPARATION OF SMALL MOLECULES****C. Belenguer-Sapiña<sup>1\*</sup>, A. Weller<sup>1</sup>, E.J. Carrasco-Correa<sup>1</sup>, P. Amorós<sup>2</sup>, J.M. Herrero-Martinez<sup>1</sup>**<sup>1</sup> Department of Analytical Chemistry, University of Valencia, Dr. Moliner 50, 46100 Burjassot, Valencia, Spain<sup>2</sup> Institut Ciència dels Materials, University of Valencia, P.O. Box 22085, 46071 Valencia, Spain

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In this work, hybrid methacrylate monolithic stationary phases were prepared by incorporation of mesoporous silica particles (MCM-41 or UVM-7) for capillary electrochromatography applications. The particles were dispersed in a polymerization mixture containing butyl methacrylate and ethylene glycol dimethacrylate as monomers, 1,4-butanediol and 1-propanol as porogens and azobisisobutyronitrile as initiator. The stability of the dispersions was investigated by UV-vis measurements at several contents of silica particles. Using continuous stirring during the capillary filling and short UV-polymerization times, polymeric beds with homogeneously dispersed mesoporous particles, specifically with contents up to 35 wt%, were prepared. The resulting hybrid monolithic columns were characterized using scanning electron microscopy. The chromatographic performance of these novel stationary phases was evaluated by using alkyl benzenes and benzoic acid derivatives as test solutes. The hybrid methacrylate polymers led to an increase both in retention as well as efficiency compared to the parent monolith due to high surface area of particles. Thus, the column efficiency reached values up to 140,000 plates m<sup>-1</sup>. The resulting hybrid monolithic columns also exhibited a satisfactory reproducibility with relative standard deviation values below 12%.

## SORCIÓN EN DISCO ROTATORIO DE FÁRMACOS ANTIDEPRESIVOS Y SU DETERMINACION POR HPLC DAD-MS/MS

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En los últimos años ha habido una creciente preocupación por parte de la comunidad científica con respecto a la presencia de contaminantes orgánicos en las aguas medioambientales, no sólo porque éstos puedan afectar a los organismos acuáticos, sino también porque pueden ser acumulados en los ecosistemas e incluso podrían representar una amenaza para la salud humana<sup>1</sup>.

Un grupo de estos contaminantes lo constituyen los Antidepresivos. Se conoce que estos fármacos son ambientalmente persistentes, pues en las plantas de tratamiento de aguas residuales no se logran eliminar completamente. Estas moléculas actúan modulando y mimetizando el efecto del neurotransmisor serotonina, el cual interviene en la regulación de una variedad de procesos fisiológicos en peces, moluscos y protozoos. Incluso a niveles de trazas presentan un marcado efecto en estos y otros organismos acuáticos<sup>3</sup>. Es por ello que resulta sumamente importante el monitoreo de estas sustancias, a partir de métodos sensibles y confiables.

La extracción en disco rotatorio (RDSE), presenta una serie de ventajas con respecto a otros métodos empleados para la extracción y preconcentración de antidepresivos en diversas matrices. En extracción en fase sólida (SPE), uno de los métodos que más se reporta con estos fines, la sorción ocurre mientras la muestra pasa unidireccionalmente a través del sólido de apoyo, mientras que la RDSE permite la recirculación de la muestra a través de la fase de extracción, maximizando así su capacidad de sorción, otra ventaja es que el disco se puede agitar a mucha más velocidad sin dañar la fase estacionaria a diferencia de cuando se emplea extracción con barra de agitación (SBSE), por lo que las velocidades de rotación con este dispositivo pueden ser más altas y de esta forma se facilita la transferencia de masas a la superficie de sorción<sup>4</sup>.

El objetivo de este trabajo fue optimizar la extracción por disco rotatorio de cuatro de los antidepresivos que más se prescriben en Chile, específicamente en la región del Bío Bío y su análisis por HPLC DAD-MS/MS.

Las condiciones optimizadas para la extracción de Venlafaxina, Fluoxetina, Escitalopram y Sertralina fueron las siguientes: fase estacionaria inmovilizada en el disco y cantidad de esta, velocidad de rotación de 3000 rpm, tiempo de extracción 105 min, volumen de la muestra 100 mL, a pH 3 cuando se extrajo con fase estacionaria SCX y 11 con C18. Para la desorción de los analitos extraídos se escogió como solvente 1mL de metanol/amoniaco 5% y metanol respectivamente. Se analizaron muestras reales tomadas del efluente y afluente de la Planta de Tratamiento de Aguas Residuales de Concepción por (LC-MS/MS), detectando la presencia de los cuatro antidepresivos.

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**SEPARATION AND DERIVATIZATION COMBINED WITH IN-TUBE SOLID PHASE MICROEXTRACTION OF ALIPHATIC AMINES**

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Amines are originated from a lot of sources, both natural and anthropogenic, and widely present in the environment. The determination of short-chain aliphatic amines, the most abundant in the atmosphere, is of great interest due to the toxicity of their degradation products (nitrosamines and nitramines) and to their contribution to secondary aerosol [1]. In-tube solid phase microextraction (IT-SPME) is a sample preparation technique that provides a very good sensitivity and selectivity, reduces the number of sample preparation steps, has very low solvent consumption, is quick and can be easily coupled on-line to HPLC. This technique has been applied to the concentration and on-line derivatization of dimethylamine (DMA) in draw/eject cycle mode [2].

On-line IT-SPME coupled to HPLC-UV is used for the concentration and separation of five aliphatic amines: methylamine (MA), dimethylamine, ethylamine (EA), diethylamine (DEA) and ethanolamine (EtA). The 9-fluorenylmethyl chloroformate (FMOC) as derivatizing agent and in-valve mode IT-SPME were chosen. The off-line derivatization (solution derivatization) is compared with on-line derivatization (inside of the extraction capillary), obtaining better results for the latter. The reagents are sequentially passed through the capillary and the best performance is obtained for the simultaneous extraction and derivatization of the amines after the extraction of the derivatization reagent. An intermediate polarity PLOT capillary provides higher concentration than a high polarity FSOT.

Several variables were optimized, including buffer concentration and pH, concentration of FMOC and its water content, conditioning of the capillary and the volumes of the reagents. A direct addition of the borate buffer to the sample was also evaluated. The best conditions involve passing sequentially through the PLOT capillary 60  $\mu$ L of acetonitrile for cleaning and conditioning, 60  $\mu$ L of borate buffer 0.15 M pH = 11.5, 120  $\mu$ L of FMOC 1 mM, 60  $\mu$ L of aqueous sample and finally 60  $\mu$ L of Milli-Q water for displacing. The higher analytical response is obtained for DMA followed by the primary amines MA and EA.

The chromatographic separation of the derivatives has been optimized using a monolithic  $C_{18}$  column and elution gradient of acetonitrile/water as mobile phase. A very rapid separation is achieved in a run time of 7.5 min, avoiding the interference of the derivatization by-products. Preliminary estimations of detection limits (around 20 ng mL<sup>-1</sup> with UV detection) are promising for the application to environmental samples and can be improved by using fluorescence detection.

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**Acknowledgements**

The authors are grateful to Xunta de Galicia (10MSD164019PR, GRC2013-047) and Ministerio de Ciencia e Innovación (CGL2010-18145). M. F. A. is grateful to the FPU Program for her grant (AP2012-5486).

## INTERPRETIVE OPTIMIZATION OF GRADIENTS OF ORGANIC SOLVENT IN MICELLAR LIQUID CHROMATOGRAPHY

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Micellar liquid chromatography (MLC) is a reversed-phase mode with mobile phases containing an organic solvent and a micellized surfactant. Most procedures developed in MLC are implemented in the isocratic mode, since the general elution problem of RPLC (the strong dependence of the retention with the solute polarity) is weaker. However, gradient elution may be still useful in MLC to analyze mixtures of compounds within a wide range of polarities, decreasing the analysis time. Also, it benefits the determination of moderately to low polar compounds in physiological fluids performing their direct injection: an initial micellar eluent with a low organic solvent content, or a pure micellar (without organic solvent) solution with a fixed amount of surfactant above the critical micellar concentration, will provide better protection of the column against the proteins in the physiological fluid. Once the proteins are swept away, the elution strength can be increased using a positive gradient of organic solvent (linear or multi-linear) to reduce the analysis time. This gives rise to the transition from the micellar mode to the submicellar mode, since micelles are destroyed at sufficiently high concentration of organic solvent. Finding the best gradient can be assisted using an interpretive optimization protocol. In previous work, the commercial DryLab® software was used to optimise gradients of organic solvent, providing satisfactory predictions. Nevertheless, it should be considered that this software was designed for eluents containing only water and organic solvent, and the interaction with the surfactant yields some deviations in the predictions. In this work, we have developed a software dedicated to aqueous-organic mobile phases containing surfactant in either micellar or submicellar conditions. The procedure was applied to the screening of  $\beta$ -adrenoceptor antagonists in urine samples, using aqueous mobile phases with sodium dodecyl sulphate and propanol.

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## **JORNADA ESPECIACIÓN**





## **COMUNICACIONES ORALES**

**ESPECIACIÓN DE ARSÉNICO EN FRESAS ENRIQUECIDAS MEDIANTE HPLC-HG-AFS****D. Sánchez-Rodas<sup>1,2,\*</sup>, I. Giráldez<sup>1</sup>, F. Martínez<sup>3</sup>, P. Palencia<sup>4</sup>, A. González de la Torres<sup>2</sup>**

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El arsénico (As) es un elemento tóxico que afecta a la salud, y que puede encontrarse en el medio ambiente en forma de especies inorgánicas con estado de oxidación +3 (arsenito As(III)), o estado de oxidación +5 (arsenato As(V)). Además de las especies inorgánicas, pueden producirse especies orgánicas que son siempre el resultado de la bioquímica de los seres vivos, por lo que es frecuente encontrar especies metiladas (p. ej. metil y dimetil arsénico (MMA y DMA)) en aguas o en seres vivos, tanto en animales como en plantas.

El cultivo de fresa es de especial importancia para la provincia de Huelva, por lo que se ha estudiado el comportamiento de estas plantas frente a una posible incorporación de arsénico a través del agua de riego, ya que tanto el As(III) como el As(V) pueden estar presentes en agua subterráneas y superficiales [1]. Para ello se ha realizado un cultivo hidropónico de fresas sobre sustrato de fibra de coco, añadiendo As(III) o As(V) en el riego durante cuatro semanas, a concentraciones de 10, 100 y 1000 ppb.

Cada semana se cogieron frutos que fueron liofilizados, triturados y homogeneizados, y se determinó su contenido total de As mediante HG-AFS. Igualmente, se determinó el contenido de As en la raíz y en el tallo de las plantas al finalizar la experiencia. De manera simultánea se realizó un estudio de especiación en los frutos mediante extracción con agua, limpieza con cartuchos C-18 y análisis mediante HPLC-HG-AFS.

Los resultados mostraron que el contenido de As en las fresas aumentó durante las cuatro semanas sólo cuando se expuso a concentraciones de 1000 ppb, siendo mayor la acumulación con As(III) (0.3 ppm) que con As(V) (0.2 ppm). La acumulación en las raíces y en los tallos de las plantas fue siempre mayor con exposición a As(III) que con As(V), encontrándose una mayor acumulación cuanto mayor concentración de As aplicado. Al final la experiencia, la concentración en la raíz (4 ppm), fue mayor que en el tallo (1.2 ppm), a su vez mayor que en los frutos.

Los resultados de especiación en los frutos indicaron la presencia mayoritaria de As(III) a lo largo de la experiencia, al tratar las plantas tanto con As(III) como con As(V). Al final de las cuatro semanas se detectó también una pequeña acumulación de As(V) y un cierto grado de metilación con la aparición minoritaria tanto de MMA como de DMA.

El presente estudio ha sido financiado gracias al Proyecto de Excelencia "Biosíntesis de compuestos de selenio y arsénico en cultivo de fresas (*Fragaria x ananassa* Duch) FQM-752 de la Consejería de Economía, Innovación, Ciencia y Empleo de la Junta de Andalucía.

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**ESPECIACIÓN DE SELENIO EN MUESTRAS DE PEZ ESPADA Y SALMÓN, MEDIANTE HPLC-ICP-MS. INFLUENCIA DEL PROCESO DE COCINADO**

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El selenio es un elemento de los considerados esenciales, sin embargo el intervalo en el que deja de ser esencial para convertirse en tóxico es muy estrecho, ya que la ingesta diaria recomendada es de  $55 \mu\text{g}\cdot\text{día}^{-1}$  y el límite en el que empieza a ser perjudicial es de  $400 \mu\text{g}\cdot\text{día}^{-1}$  [1]. Aun así, sus propiedades dependen fundamentalmente de su forma química, siendo las más abundantes Se(IV), Se(VI), SeMet, SeMetSeCys y SeCys. Las especies más tóxicas son las inorgánicas, mientras que algunas de las orgánicas son selenoproteínas, como es el caso de la selenocisteína, SeCys, o son proteínas con selenio, como es la selenometionina, que se produce gracias a la capacidad que tiene este elemento de sustituir el azufre en biomoléculas por la similitud entre ambos. Las selenoproteínas tienen un papel importante en el metabolismo y su deficiencia puede dar lugar a problemas para la salud [2]. En numerosos países existe esta deficiencia de selenio entre la población, y para combatirla nuestro organismo necesita obtenerlo de una forma eficiente, como puede ser a través de la dieta [3]. Uno de los alimentos con mayor contenido de selenio en nuestra dieta son los pescados, en especial aquellos que están en lo alto de la cadena trófica (atún, pez espada, etc.) [4]. Además, el conocimiento de las especies de selenio que aporta el alimento es de suma importancia, ya que tienen propiedades y funciones muy diferentes entre sí. Por otro lado, la ingesta de este tipo de alimentos implica, en la mayoría de los casos, su previo cocinado, con lo cual es crucial entonces conocer tanto si el contenido de selenio varía respecto al original, así como si las especies originales sufren transformaciones tras dichos procesos [5].

En el presente trabajo se ha llevado a cabo la determinación de selenio y sus especies en dos alimentos frecuentes en nuestra dieta, como son el pez espada y el salmón, tanto crudos como cocinados (a la plancha y horneados), y en el caso del salmón se estudiaron ejemplares salvajes y criados en piscifactoría, ya que ambos difieren notablemente en su propia dieta. Para la consecución de estos objetivos, se ha optimizado el proceso de extracción de las especies de Se en este tipo de alimentos, y se ha hecho a través de una digestión enzimática con Proteasa XIV, utilizando una sonda de ultrasonidos, en las matrices indicadas. Se ha desarrollado un diseño experimental para este proceso de extracción, siendo las variables a tener en cuenta el tiempo de extracción, la potencia de la sonda y la cantidad de proteasa utilizada. Asimismo, se evaluó la eficacia de dicha extracción partiendo de la muestra de pescado fresco o previamente sometido a una etapa de secado. El contenido de selenio total encontrado, analizado por la técnica de ICP-MS, fue de 0.68 mg de selenio por kg de pescado fresco, para el pez espada, y de  $0.52 \text{ mg kg}^{-1}$  y  $0.39 \text{ mg kg}^{-1}$  para el salmón salvaje o de piscifactoría, respectivamente, referido igualmente a pescado fresco. La extracción de las especies fue del 100% en todos los pescados frescos cuando se emplearon 6 min de sonda a 60% de potencia y con 20 mg de la proteasa. Sin embargo, una vez cocinados, esa extracción disminuía hasta el 50% en el caso del salmón de piscifactoría. El análisis de las especies se realizó por cromatografía de líquidos con columna de intercambio aniónico (PRP-X100) acoplada al ICP-MS, encontrándose que la especie predominante, tanto en el pez espada como en ambos tipos de salmón, fue la selenometionina (SeMet). El proceso de cocinado de los pescados nos llevaba a una reducción de casi un 50% en el contenido de dicha especie.

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## ARSENIC SPECIATION IN EDIBLE VEGETABLES FROM NORTHERN CHILE

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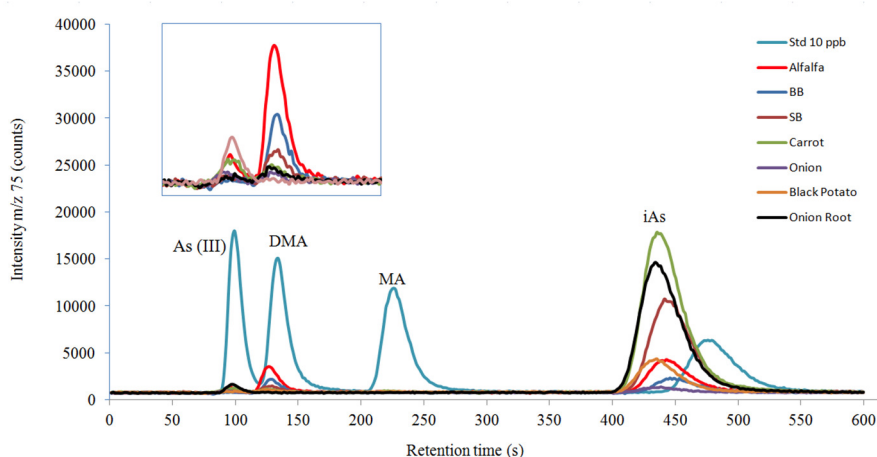
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Arsenic contamination is a well-known problem especially in South East Asia and South America where people is exposed to high concentration levels of this element in soil and water.

In the case of Northern Chile, the high contents of As in the environment are due to both natural and anthropogenic sources. The naturally-occurring As in Chile is mostly related to the volcanic activity, while the emission from copper smelting process becomes one of the As anthropogenic sources especially in the Antofagasta region. The monitoring of As content in terrestrial plants can provide valuable information on As level contained in the environment where they are cultivated, and on the levels of population exposure through the food chain [1].

In this work, different plant matrices from agricultural villages located at the north of Chile (Chiu Chiu, Caspana and Socaire) were sampled and total As content and species were analysed. Studied matrices were carrot, onion, garlic, black potato, broad and skin from beans, and also alfalfa was considered as animal feed. Total As content was analysed by HG-AFS and ICP-MS after a microwave digestion, whereas for speciation an HPLC-ICP-MS method based on anionic and cationic exchange columns was used for measuring the corresponding extracts. For quality control purposes, a certified reference material ERM-BC 211 (Rice) and a reference material IPE 990 WEPAL (Alfalfa) were used. Additionally, for speciation, extraction efficiency and column recovery were also calculated.

Total As content in the analysed plants ranged from 44 to 9500  $\mu\text{g}\cdot\text{kg}^{-1}$ , which overcome the contents reported for terrestrial plants grown in non-contaminated soils. For onion, garlic and broad bean, As contents were mostly found in roots and skin. Speciation results showed that inorganic arsenic (iAs) is the dominating species in all matrices (> 50%) whereas dimethylarsenic acid (DMA) was found at relatively low level (1.75 – 33%) for most of the matrices except the onion. Other unidentified species were also present in the extracts as minor constituents.



Chromatogram of arsenic species obtained in edible vegetables (anionic exchange column)

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**SIMULTANEOUS DETERMINATION AND SPECIATION ANALYSIS OF ARSENIC AND CHROMIUM IN IRON SUPPLEMENTS USED FOR IRON-DEFICIENCY ANEMIA TREATMENT BY HPLC-ICP-MS**

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This work proposes the use of high performance liquid chromatography coupled to inductively coupled plasma mass spectrometry (HPLC-ICP-MS) for simultaneous speciation of arsenic and chromium in iron supplements used for the treatment of anemia. The sample preparation procedure recommended for the total determination of arsenic and chromium was established using acid digestion in a microwave assisted oven. For speciation analysis, however, the microwave-assisted extraction procedure involved the use of water as extraction solvent at 90°C for 30 minutes. This method was based on the procedure developed by Narukawa et al. [1]. The chromatographic separation was performed using a mobile phase containing 1.0 mM tetrabutylammonium hydroxide (TBAH), 0.7 mM ethylenediaminetetraacetic acid (EDTA) and 5% methanol at pH 7.2. Helium was used in the collision cell for elimination of the interferences. Under optimized conditions, the separation and detection of the As(III), As(V), Cr(III) and Cr(VI) species can be performed in 5 minutes. The external calibration technique was used, with standards prepared in the mobile phase in concentrations ranging from 0 to 80 µg L<sup>-1</sup> for As(III) and As(V), and from 0 to 400 µg L<sup>-1</sup> for Cr(III) and Cr(V). The limits of quantification obtained were 0.008, 0.010, 0.5 and 0.14 µg g<sup>-1</sup>, for As(III), As(V), Cr(III) and Cr(VI), respectively. The accuracy of the method was evaluated and confirmed by addition/recovery tests. The recoveries obtained varied from 81 to 110%. The proposed method was applied to the speciation analysis of arsenic and chromium in commercially available iron supplements acquired in several cities in Brazil and Spain. The content of the species ranged from 0.01 to 1.3 µg g<sup>-1</sup> for arsenic, and from 0.4 to 61.2 µg g<sup>-1</sup> for chromium.

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**Acknowledgements.** The authors are grateful to Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq, Grant Numbers: 202192/2014-8 and 161456/2015-4) and to Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES-PROAP 2015) for providing grants and fellowships and for financial support. Authors also thank Xunta de Galicia (Grupo de Referencia Competitivo 6RC2014/2016) for financial support.

## ANTIMONY SPECIATION IN SPIRITS STORED IN PET CONTAINERS

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Polyethylene terephthalate (PET) is the fastest growing plastic used for food packaging applications as a result of its popular use for replacing glass containers. Food packaging has the main function of protecting food from contamination which requires that the packaging material is inert enough to not cause food contamination during the entire contact period. For this reason the presence of chemicals, such as antimony, present in the packaging material need to be studied in detail. Even if spirits are mainly bottled in glass, in some countries is typical to store local homemade spirits in PET bottles.

In this work, total and species of antimony were determined in several PET bottled Greek raki and tsipouro. Additionally, Sb migration behaviour at elevated storage temperature in raki samples has also been investigated. Direct analysis of samples by using ICP-MS provided total Sb concentrations between 0.4 – 4 µg L<sup>-1</sup> which are higher than those reported in water samples stored in PET [1] probably due to the high ethanol content as well as the presence of potential organic ligands that may be promoting Sb extraction.

Antimony speciation analysis by LC-ICP-MS was also assessed, showing the presence of inorganic Sb species (non-complexed Sb(V) and Sb(III)) along with an unknown Sb complex, which was the predominant species in all samples analysed. In order to determine the structure of this complex, several studies by using liquid chromatography with high-resolution tandem mass spectrometry were performed. The analysis gave evidence for an acetaldehyde-bisulphite pyruvate Sb complex with the formula C<sub>7</sub>H<sub>14</sub>O<sub>12</sub>S<sub>2</sub>Sb, with ligands expected to be present in the raki matrix.

The influence of storage time and storage temperature at 60 °C on Sb migration in six raki samples was also assessed. Both total Sb and speciation analysis was carried out by ICP-MS and LC-ICP-MS, respectively. The Sb content was determined at the beginning of the experiment, after 7 and 15 days of storage and concentration levels increased with time up to 30 – 50 µg L<sup>-1</sup> at the end of the period. Regarding speciation, concentration levels of the new identified Sb complex decreased slightly or even disappeared in some samples whereas Sb(V) concentration increased slightly in all samples (0.8 – 26 µg L<sup>-1</sup>) whereas that of Sb(III) increased sharply already after a week of storage (2.9 – 11.6 µg L<sup>-1</sup>). It is therefore highly recommended not to expose PET bottled beverages to elevated temperatures, as a rapid release of Sb has been demonstrated. The results obtained in this study could be used in the proposal of further Directives on Sb in other beverages other than from water.

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**A UNIVERSAL SOLUTION TO STANDARDIZE ABSOLUTE QUANTIFICATION OF BIOMOLECULES USING HPLC WITH ICP-MS DETECTION****Francisco Calderón Celis, Alfredo Sanz-Medel, Jorge Ruiz Encinar**

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The determination of absolute levels or quantities of a target biomolecule is currently one of the main challenges in life sciences. Such quantitative info can boost the discovery of new biomarkers, the understanding of systems biology or compounds toxicity, as well to quantitatively characterize standards used in metrology or in quantitative applications. During the last decades, ICP-MS has been established as an effective tool for absolute quantification of biomolecules needless of specific standards, as it is the case of more extended molecular MS approaches. ICP-MS hence enables one to quantify almost any element, naturally present or tagged to the biomolecule of interest, independently of the species and molecular environment [1].

However, the standardization and universal applicability of HPLC-ICP-MS for the quantitative speciation of biomolecules is hampered by the effect of carbon-containing sources. Carbon influences the ionization processes of the heteroatoms in the plasma, and this effect depends on the amount of carbon reaching the plasma. Therefore, elemental response factors in chromatographic analysis are usually different depending on the elution time from the chromatographic column [2]. Correction of this carbon effect has been commonly achieved with the use of organic sheath flows, or *on-line* isotope dilution (in the case of multi-isotopic heteroatoms). These approaches, nonetheless, entail complex instrumentation set-ups, specific conditions for each heteroatom, and in the case of isotope dilution it specifically requires for isotopically enriched standards.

In this case, we have approached the correction of such effect by means of the direct addition of methane gas to the ICP-MS. This addition leads to carbon-saturated plasma so as to screen sensitivity variations caused by the organic solvents variations (gradient) during chromatographic separations prior to the detection. Systematic studies on how the addition of methane affects sensitivity variations and also detection limits were carried out. It was evaluated for six of the most abundant heteroelements (S, P, As, Br, Se, and I) present in biomolecules of great relevance in life sciences such as (phosphor/seleno)proteins, drugs, metabolites, pesticides, etc.

Optimized conditions found enabled the simultaneous detection of such elements with constant response factors along the whole LC-ICP-MS analysis. As a proof of concept, simultaneous quantification of S-, Se-, and Br-containing biomolecules was achieved just using pure non-specific standards that contained the target heteroatoms as generic standards. Results obtained were validated with isotope dilution analysis.

The proposed approach aims to become a universal method for the quantification of any biomolecule containing an ICP-detectable heteroatom without requiring for specific standards, conditions or instrumentation.

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**CUANTIFICACIÓN ABSOLUTA DE PROTEÍNAS MEDIANTE ICP-MS SIN NECESIDAD DE PATRONES ESPECÍFICOS: APLICACIÓN A LA CARACTERIZACIÓN CUANTITATIVA DE VENENOS DE SERPIENTE.**

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La proteómica cuantitativa se ha convertido en uno de los principales focos de interés de la última década. Este tipo de análisis permite un mejor entendimiento de los sistemas biológicos, ya que proporciona información más completa acerca de la organización y dinámica celular. Además, podría tener un gran potencial en la validación de posibles biomarcadores de enfermedades.

La técnica analítica por excelencia en proteómica cuantitativa es la espectrometría de masas, ya que proporciona versatilidad, y una mejora en la precisión y sensibilidad en comparación con metodologías tradicionales (generalmente menos específicas y más laboriosas). Los principales enfoques basados en la MS en cuantificación absoluta de proteínas (AQUA, QconCAT) emplean análogos del péptido o proteína diana marcados con isótopos estables. Sin embargo, una de las principales limitaciones de estos enfoques es la necesidad de sintetizar patrones enriquecidos específicos para cada proteína y su correspondiente certificación. Por lo tanto, existe una urgente necesidad de desarrollar metodologías sencillas y rápidas que permitan la cuantificación absoluta de proteínas sin necesidad de patrones específicos.

En este trabajo, se propone una metodología híbrida basada en cromatografía líquida capilar acoplada a espectrometría de masas con plasma de acoplamiento inductivo (HPLC-ICP-MS) para la cuantificación absoluta de proteínas empleando patrones genéricos. La cuantificación se lleva a cabo a través de la medida elemental del azufre, que se encuentra de forma natural en las proteínas, y empleando dilución isotópica post-columna [1].

Inicialmente, se validó la metodología con patrones de proteínas. Posteriormente, se aplicó a la cuantificación absoluta de las proteínas presentes en muestras reales de venenos de serpiente (venómica). El estudio del proteoma de los venenos permite el desarrollo de nuevos fármacos, así como el conocimiento de la composición y la abundancia de las distintas proteínas que conforman el veneno, lo cual es importante para la comprensión y tratamiento de los síntomas del envenenamiento.

La combinación de la cuantificación proporcionada por HPLC-ICP-MS con la identificación proporcionada por la espectrometría de masas molecular con fuente de electrospray (ESI-MS) permitió traducir los pmol de S cuantificados en cada pico cromatográfico en pmol de las diferentes proteínas individuales presentes en el veneno. Los resultados obtenidos para 4 venenos de serpiente diferentes fueron comparados críticamente con los obtenidos previamente empleando otras estrategias más habituales en proteómica [2].

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**DETECCIÓN, CARACTERIZACIÓN Y CUANTIFICACIÓN DE NANOPARTÍCULAS DE  
ÓXIDO DE TITANIO EN MUESTRAS COMPLEJAS MEDIANTE AF4-ICPMS**

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Uno de los casos más representativos del actual auge de los nanomateriales, en cuanto a su incorporación en una gran diversidad de productos de consumo, es el dióxido de titanio (TiO<sub>2</sub>). Debido a su poder refractario en su forma nanoparticulada (1-100 nm), muchas de sus aplicaciones están relacionadas con productos comerciales como cremas solares, cosméticos, envases y recubrimientos, siendo el campo de la fotocatalisis uno de los más importantes. Además, a este amplio rango hay que añadirle su uso en remediación medioambiental, como aditivo alimentario y como pigmento blanqueador. Conscientes de su relevancia, el interés por estudiar sus posibles efectos potenciales tanto en la salud humana como el medio ambiente ha aumentado.

Debido a la disparidad de tipos de muestras y posibles escenarios en los que encuentren presentes estos nanomateriales, se hace patente la necesidad del desarrollo de metodologías que puedan aportar información adicional y complementaria a las técnicas que se emplean habitualmente como las distintas microscopías electrónicas o las basadas en la dispersión de radiación.

En este trabajo se plantea el uso de la técnica de Fraccionamiento en Flujo mediante Campos de Flujo Asimétrico (AF4) acoplado a absorción molecular en ultravioleta-visible (UV-Vis) y espectrometría de masas con plasma de acoplamiento inductivo (ICP-MS) para la detección y caracterización de nanopartículas (NPs) de TiO<sub>2</sub> en diversas muestras complejas.

Para ello, se ha llevado a cabo en primer lugar un estudio de las diferentes variables que determinan la separación en AF4, tales como la estabilización de las nanopartículas, fundamentalmente a través de la composición del portador, o la resolución de suspensiones de NPs de diferentes tamaños, mediante el uso de diferentes caudales para su correcta elución en el canal. Se presentarán resultados en los que, por primera vez, se evalúa el uso de suspensiones estándar de NPs de TiO<sub>2</sub> de tamaño conocido para calibrar el canal y caracterizar el tamaño de NPs de TiO<sub>2</sub> presentes en diversas muestras, con el objetivo de compensar posibles diferencias en la elución que se puedan producir con el uso de otros estándares (látex o poliestireno). Las condiciones de separación escogidas han permitido obtener además elevadas recuperaciones (del orden del 90%), con unos límites de detección para AF4-ICPMS de 0,88 µg/L en Ti. Finalmente, el desarrollo de toda esta metodología se ha llevado a la práctica en la detección y caracterización de NPs de TiO<sub>2</sub> en distintos tipos de muestras.

Este trabajo ha sido subvencionado por el proyecto CTQ2015-68094 del Ministerio de Economía y Competitividad (MINECO) de España.

**COMBINACIÓN DE LA EXTRACCIÓN EN PUNTO DE NUBE CON TXRF Y SP-ICPMS PARA LA DETERMINACIÓN DE NANOPARTÍCULAS DE PLATA EN MUESTRAS ACUOSAS****L. Torrent <sup>(1)</sup>, F. Laborda <sup>(2)</sup>, M. Iglesias <sup>(1)</sup>, E. Marguí <sup>(1)</sup>, M. Hidalgo <sup>(1)</sup>**

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En los últimos años se ha incrementado la producción de nanopartículas metálicas en la industria, siendo las nanopartículas de plata (AgNPs) ampliamente usadas por sus propiedades antibacterianas. La expansión de su fabricación provoca inevitablemente su liberación en el medio ambiente generando una preocupación por los efectos que puedan tener sobre el medio y los organismos biológicos. En el medio estas partículas son susceptibles de sufrir procesos de transformación como la disolución a Ag<sup>+</sup> que esta relacionada con la toxicidad de las AgNPs [1]. Debido a la coexistencia de Ag<sup>+</sup> y AgNPs es necesario el desarrollo de métodos analíticos para el análisis de especiación.

La extracción en punto de nube, también conocida como “cloud-point extraction” (CPE), permite la separación y preconcentración de AgNPs de la Ag<sup>+</sup> sin alterar ni la forma ni el tamaño de éstas [2]. Esta metodología se combina principalmente con la espectrometría de masas con plasma de acoplamiento inductivo con una previa digestión mediante microondas del extracto. El objetivo de la presente contribución es evaluar la posibilidad de combinar el CPE con otras técnicas analíticas evitando la etapa de digestión. Con este objetivo se estudiaron las ventajas e inconvenientes de la combinación del CPE con la espectrometría de fluorescencia de rayos-X por reflexión total (TXRF) y la espectrometría de masas con plasma de acoplamiento inductivo de una sola partícula (SP-ICPMS) para la identificación, caracterización y cuantificación de AgNPs en muestras acuosas.

En primer lugar, se establecieron las condiciones idóneas para la separación y preconcentración de AgNPs de distintos recubrimientos y tamaños mediante CPE. Posteriormente, se determinó el tratamiento del extracto CPE más adecuado para su análisis directo y las mejores condiciones de análisis tanto para TXRF como para SP-ICPMS.

Para el análisis por TXRF, el extracto orgánico rico en AgNPs se evaporó y reconstituyó en tetracloruro de carbono (20 µL) previamente a su análisis. Usando un volumen de deposición de 5 µL y un tiempo de medida de 2000 s fue posible la cuantificación de Ag total (detección Ag-K $\alpha$ ) a niveles de concentración de µg·L<sup>-1</sup> en muestras acuosas. Aunque los límites de detección de esta técnica son altos comparados con otras técnicas espectroscópicas, como ICPMS, no se requiere el uso de una gran cantidad de reactivos, el volumen de muestra para llevar a cabo el análisis es pequeño y es una técnica económica debido a que no necesita fungible.

Para el análisis por SP-ICPMS, el extracto CPE se diluyó con una solución de glicerol 1% a una concentración adecuada (ng·L<sup>-1</sup>). Con un tiempo de medida de 5 ms se obtuvo información sobre la presencia de AgNPs, la concentración en número de partículas de Ag y el tamaño de partícula. A pesar de la necesidad de volúmenes mayores en SP-ICPMS en comparación con la TXRF, los límites de detección son muy inferiores y permite la obtención de información cualitativa y cuantitativa de AgNPs en el extracto CPE de una muestra acuosa.

En conclusión, a pesar de las ventajas e inconvenientes de ambas técnicas de análisis, la combinación de TXRF y SP-ICPMS con CPE puede ser beneficiosa para obtener información complementaria de AgNPs en muestras acuosas.

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## **COMUNICACIONES FORMATO PÓSTER**

## ESPECIACIÓN DE SELENIO EN FRESAS ENRIQUECIDAS MEDIANTE HPLC-TR-HG-AFS

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El selenio (Se) es un elemento esencial necesario para la dieta humana, al que se le atribuyen efectos beneficiosos por su actividad antioxidantes. Es por ello que existen estudios sobre enriquecimiento de vegetales con Se para poderlos emplear como una fuente de este elemento, ya que se produce la acumulación y la biotransformación en selenoaminoácidos que son fácilmente asimilable por el organismo humano.

Se ha llevado a cabo un estudio de enriquecimiento de Se en plantas de fresas (uno de los principales cultivos agrícolas de la provincia de Huelva), en cultivo de invernadero a lo largo de siete semanas. El cultivo se realizó en modo hidropónico, empleando fibra de coco como sustrato. Durante la experiencia se aplicó Se inorgánico (selenito Se(IV) y selenato (Se(VI)), y seleno-metionina (SeMet) por vía foliar o por riego en raíz, con concentraciones comprendidas entre 0.5 y 500 mg L<sup>-1</sup>.

Los frutos fueron recogidos y liofilizados a lo largo de la experiencia. Posteriormente fueron triturados y homogeneizados, y sometidos a tratamiento enzimático con una mezcla acuosa de proteasa XVI:lipasa (1:2) y sonda de ultrasonidos. El extracto fue centrifugado y sometido a análisis de especiación con cromatografía de alta resolución, termoreducción, generación de hidruros y detección por espectroscopía de fluorescencia atómica (HPLC-TR-HG-AFS). Las selenoaminoácidos estudiados fueron seleno-metionina (SeMet), seleno-metil-selenocisteína (SeMetSeCys) y seleno-cistina (SeCyst), además de Se(IV) y Se(VI).

Las primeras experiencias con concentraciones bajas de selenio (0.5 y 5 mg L<sup>-1</sup>) indicaron una baja una baja o nula incorporación del selenio a las plantas cuando se adición en forma de Se(IV) o SeMet. Para el Se(VI) sí se observó una absorción por riego en raíz, aunque no se produjo una biotransformación apreciable en selenoaminoácidos. Una vez suspendida la aplicación de Se(VI) durante seis semanas, se constató una fuerte desacumulación del Se(VI) presente en los frutos en las dos semanas posteriores.

Respecto a la aplicación foliar, se observó también poca acumulación y transformación de las especies inorgánicas de Se a bajas concentraciones de aplicación, siendo el Se(IV) la especie mejor asimilada por la planta. Al incrementarse la concentración de Se(IV), sí se observó su biotransformación apreciable en selenoaminoácidos, principalmente SeMet [1].

El presente estudio ha sido financiado gracias al Proyecto de Excelencia "Biosíntesis de compuestos de selenio y arsénico en cultivo de fresas (*Fragaria x ananassa* Duch) FQM-752 de la Consejería de Economía, Innovación, Ciencia y Empleo de la Junta de Andalucía.

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## DETERMINATION OF METALS AND SPECIES OF ARSENIC IN RICE AND GLUTEN-FREE FOODS

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Food and drinking water are the main sources of exposure to Arsenic (As) for humans [1,2]. In this sense, the presence of arsenic in rice and its products of transformation has been demonstrated, mainly inorganic form [3,4]. This fact has important consequences in celiac people who have a rice-based diet, which determine the interest to investigate the content of arsenic species in foods without gluten. In this study, an analytical procedure designed to address this problem has been optimized and applied to different varieties of rice-based food.

For the analysis of trace metals, the samples were solubilized by acid treatment (4 ml of nitric acid) in a microwave oven. Acid speciation was performed by gentle extraction of powdered food with TFA, followed by the speciation of arsenic forms of interest (As (III), As (V), DMA and MMA) by anion exchange liquid chromatography and detection with ICP- MS (Thermo XSeries2 (Thermo Scientific)). The procedure was validated using a certified rice reference material containing arsenic species (IRMM-804).

Different foods derived from rice (without gluten) of different commercial brands were analyzed: rice, cereal, spaghetti, noodles, brown rice pancakes, cookies and muffins.

Mutielemental analysis (As, V, Cr, Co, Cu, Zn, Se, Mo, Cd, Sb, Ba and Pb) demonstrated that rice is the product with the highest amount of metals. The As speciation results showed that the most abundant form is As (III) for all rice-based foods, including the rice itself. The species As (V) and DMA present very low values in both types of food. On the other hand, the absence of MMA in samples of rice and food derived from rice was observed, which seems to indicate a migration of these species towards the husk of the cereal. All values are within the limits allowed by law.

In conclusion, the method developed for As speciation in rice-based foods provides reliable results demonstrating the higher presence of this element in rice respect other foods derived from it and that As(III) is the species most abundant, although at levels under the reference established by regulations.

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**WHAT HAPPEN WITH ARSENIC SPECIES AFTER GASTROINTESTINAL DIGESTION?****M.R. Domínguez-González, V.G. Calvo-Millán, M.C. Barciela-Alonso, P. Bermejo**

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Nowadays the total content of a toxic element, like As, in food is a poor indicator of risk assessment. Speciation studies are necessary because the toxicity of the elements depends on their concentration but also on their chemical form. The inorganic forms of arsenic are more toxic than organic forms and the toxicity increases with decreasing oxidation states ( $\text{AsH}_3 > \text{As}^{\text{III}} > \text{As}^{\text{V}} > \text{MMA} > \text{DMA}$ ). Rice plant has the ability to bioaccumulate essential and toxic trace elements such as arsenic. Children under three years old are the most exposed consumers in Europe to inorganic arsenic through the diet (rice-based food). Recently, the European Commission established the maximum levels of inorganic arsenic in foodstuffs [1]. This regulation establishes a maximum level of inorganic arsenic of 0.10 mg/Kg in rice destined for the production of food for infants and young children.

An *in vitro* digestion procedure has been applied to study if changes in As species occur during gastrointestinal digestion process. Rice samples were subjected to simulated gastric conditions (use of pepsin at pH 2.0, orbital – horizontal shaking at 150 rpm, 37°C, 2 hours) followed by a simulated intestinal digestion (use of pancreatin and bile salts at pH 7.4, orbital – horizontal shaking at 150 rpm, 37°C, 2 hours) with dialysis membranes (10 kDa). Arsenic species were determined in rice and in the dialyzate fraction by high performance liquid chromatography coupled to inductively coupled plasma mass spectrometry (HPLC-ICP-MS). A Hamilton PRP-X100 (150 x 4.1 mm, 5 µm particle size) column was used for the separation of arsenic species, using 25 mM  $\text{NH}_4\text{H}_2\text{PO}_4$  (pH 6) as mobile phase operating at a flow rate of 1 mL min<sup>-1</sup>.

The proposed method has been applied to several rice samples acquired in local market. The results obtained confirm As species transformation after *in vitro* digestion procedure.

**Acknowledgments:**

The authors wish to thank the Xunta de Galicia (Grupo de Referencia Competitivo 6RC2014/2016) for financial support. This programme is co-finance by European Union FEDER funds.

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## SPECIATION OF MERCURY IN SEAWEEDS AND MUSHROOMS

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Seaweeds begin to play an important role in the diet of Europeans, as among some of its properties we can highlight its anti-HIV effects, anti-tumor activity ... Mushrooms are also gaining importance as components of the diet due to its excellent organoleptic and nutritional properties. However, it is well known that these species can accumulate relatively high concentrations of heavy metals. According to an article published by the European Food Safety Authority (EFSA) in December 2012, a Tolerable Weekly Intake (TWI) of Methylmercury of 1.3 µg/kg body weight and for inorganic mercury of 4 µg/kg were stated. In addition to these data, social pressure, disasters already happened and the fact that seaweeds are bioindicators of pollution levels in the environment around them an interest of the scientific community in the determination of some elements such as Hg in this kind of samples have aroused. Reviewing the literature we have not found methods of MeHg speciation in seaweeds, but nevertheless we have found high levels of total Hg in some species, it would be advisable to know which species can present high concentrations. The main objective of our study is the optimization, validation and subsequent application of an analytical methodology to determine the organic mercury content in samples of seaweeds and mushrooms, which will allow us to evaluate the degree of danger of the ingestion of this kind of food for our health.

The proposed extraction procedure was based on the method of the inter-collaborative study IMEP-115, backed by Regulations 1881/2006 and 882/2004, validated and applied to fish samples in the Department of Analytical Chemistry of the University of Valencia. The extraction was based on the use of organic solvent, which extracts the organic mercury and then it was back extracted using an L-cysteine solution; this last was introduced in the direct mercury analyser when Hg was determined by atomic absorption. The seaweed samples presented concentrations of total Hg from not detected up to 58 µg/kg, whereas in the samples of mushrooms the concentration oscillates between 8.4 and 2401 µg/kg. It is important to say that the levels of total Hg in the samples of *Bolletus edulis* (*Bolletus edulis*) and dried *Niscaleo* (*Lactarius deliciosus*) were higher than in the other samples. In the case of "rebollones" all samples had the same order of Hg concentration (284-674 µg/kg). In this way we can concluded that the total amount of Hg in seaweeds was very low and does not represent a health risk. The organic Hg contents of the seaweeds, mushrooms and "rebollones" samples were very low, below the limit of detection of the methodology tested even increasing the sample size. The contents of total Hg were of the same order as those published in the bibliography.

Acknowledgment: authors acknowledge the financial support of the Generalitat Valenciana project PROMETEO II 2014-077.



EVALUATION OF IODINE BIOAVAILABILITY IN SEAWEED USING *IN VITRO* METHODS

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Iodine is an essential element, necessary for synthesis of the thyroid hormones. Deficiencies in iodine, which affects about two thousand million people worldwide, cause endemic goitre, the most visible sign, and central nervous system, damage causing mental retardation in children<sup>1</sup>. Most studies identify deficiencies in iodine intake, however, in some cases high intakes above the recommended values have also been described. In certain susceptible population groups, ingestion of high iodine quantities might increase the risk of developing iodine-induced thyroid dysfunction.

Food is the major source of iodine for the general population, being seafood products the type of food with the highest content. Seaweeds have an inherent biologic capacity to concentrate iodine from the sea, consequently, concentrations up to 6138 mg/kg have been found in commercialized samples<sup>2,3</sup>. Excessive intake of iodine can occur as a result of ingestion of large amounts of seaweed. However, the food after ingestion undergoes a series of processes that can modify the quantity of iodine that reaches the systemic circulation (bioavailability). Studies on the bioavailability of iodine from food are scarce and indicate that the bioavailable amount is generally lower than ingested<sup>2,4,5</sup>.

The aim of this study is to estimate the bioavailability of iodine present in different types of seaweed. Thus, iodine *in vitro* bioavailability estimation from different commercialized seaweed has been studied using different *in vitro* approaches (solubility, dialyzability and transport and uptake by intestinal cells). Results obtained indicate that iodine is available after gastrointestinal digestion for absorption (bioaccessibility: 49-82%), being Kombu the seaweed with the highest bioaccessibilities. Nevertheless the use of dialysis membranes and cell cultures (co-cultures Caco-2/HT29-MTX) to elucidate bioavailability modifies the estimation of iodine that may reach the systemic circulation (dialysis: 5-28%; cell culture:  $\leq 3\%$ ), showing a low exposure to iodine after seaweed intake.

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**FULL VALIDATION OF AN ANALYTICAL METHOD FOR THE DETERMINATION OF INORGANIC ARSENIC IN SEAFOOD**

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Interest in the determination of inorganic arsenic (iAs) in products for human consumption such as food commodities, rice, seafood, etc., among others, is driven by the wide recognition of its toxic effects on humans, even at low concentrations. Currently, the need for robust and reliable analytical methods is recognized by various international safety and health agencies, and by organizations in charge of establishing acceptable tolerance levels of iAs in food. Very recently, the European Union published Regulation (EU) 2015/1006 amending Annex to Regulation (EC) No 1881/2006 regarding the maximum levels of iAs in foodstuffs, especially rice and rice-based products. Furthermore, EFSA and JECFA highlighted the need for robust, validated analytical methods for the determination of iAs in a range of food items; and the need for certified reference materials (CRMs) for iAs.

In this work, an analytical method for the determination of iAs in different types of seafood is developed and validated. Fish and shellfish are of interest because of their high presence in our diet and their high arsenic content. The extraction is performed by using nitric acid 0.2% and hydrogen peroxide 1%. The system LC-ICP/MS is used for measurements. Total arsenic is also determined by microwave digestion and inductively coupled plasma mass spectrometry (ICP/MS).

The main quality parameters: LOQs, Linearity, intermediate reproducibility (according to ISO 5725-3), accuracy, trueness as well as expanded uncertainty are assessed for iAs. TORT-2 certified reference material was used throughout the study for checking the accuracy, showing good agreement with the results on arsenic species reported in the literature.

# SPECIATION OF ORGANOMANGANESE AND INORGANIC MANGANESE IN WATER SAMPLES BY HEADSPACE THIN-FILM MICROEXTRACTION ONTO GRAPHENE MEMBRANES

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The determination of inorganic and organic manganese compounds, such as Mn(II) and methyl(cyclopentadienyl)-tricarbonyl manganese (MMT), would be an important achievement from the point of view of bioavailability [1]. Mn(II) occurs naturally in surface and groundwater (e.g. seawater and freshwater). On the contrary, MMT can occur in water through anthropogenic pollution. MMT is a highly toxic organometallic compound by different exposure routes, *i.e.* inhalation, skin adsorption and ingestion. Although MMT is rapidly photolyzed by sunlight in the atmosphere (with a half-time less than 2 min), the rate of degradation in natural waters and sediments is very slow, with half lives ranging from 0.2 to 1.5 years [2]. Until now different analytical approaches, mainly based on chromatographic techniques, have been developed for the speciation analysis of Mn. A step forward is to develop non-chromatographic approaches for Mn speciation providing simpler and less expensive ways to perform analysis. Since MMT is expected to be at low concentrations in environmental samples, a preconcentration step before analysis would be necessary. With this purpose, nanoparticles (NPs) are presented as powerful tools for extraction techniques due to their large surface area and fast sorption kinetics [3]. Besides, NPs allow the miniaturization of experimental setups typically used for extraction and pre-concentration using solid phases, hence resulting in low sample/reagent consumption and generation of minimal wastes. Among the different type of NPs, graphene and graphene oxide (GO) have recently attracted much attention as new sorptive materials [4]. Most reported applications using carbon nanomaterials are based on dispersive micro-solid phase extraction strategy, where NPs are isolated by filtration or centrifugation and an elution step is necessary before measurement. New formats of carbon nanomaterials, such as graphene membranes, would simplify the extraction procedure thus avoiding separation steps. Furthermore, direct measurement by total reflection X-ray fluorescence (TXRF) could be performed without the need for an elution step before analysis. In the present work a novel approach for speciation of MMT and Mn(II) in water samples based on head-space thin film microextraction (HS-TFME) onto unmodified graphene membranes followed by direct TXRF analysis is described [5]. Unmodified graphene membranes ( $\varnothing$  10 mm) were synthesized by means of drop-casting of GO onto glass substrates followed mild thermal reduction. When a water sample containing MMT and Mn(II) is heated (at 55°C) only MMT is transported to the headspace being retained by  $\pi$ - $\pi$  stacking interaction with the delocalized  $\pi$ -electrons in the surface of the graphene membrane. Selective extraction and preconcentration of MMT is achieved, with a detection limit of 18 ng L<sup>-1</sup> MMT. For the detection of the total Mn, and aliquot of the water sample is deposited onto a quartz substrate and directly measured by TXRF. The inorganic Mn content (as Mn(II)) could be calculated as the difference between the total Mn and MMT contents. Graphene membranes proved to be excellent substrates for TXRF owing to their thickness at nanoscale level, high sorptive capacity and low background spectra. In addition, compared to hyphenated techniques (e.g. gas chromatography-mass spectrometry, GC-MS), TXRF benchtop systems which use air-cooled low-power X-ray tubes and silicon drift detectors, requires neither inert gasses or cooling water consumption, thus providing simple, rapid and cost-effective approach for Mn speciation.

*Acknowledgements:* Financial support from the Spanish Ministry of Economy and Competitiveness (Project CTQ2015-68146-P) (MINECO/FEDER) is gratefully acknowledged.

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## BIOMONITORIZACIÓN DE ESPECIES DE ARSÉNICO EN ORINA EN LA POBLACIÓN ADULTA DE ANDALUCÍA

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El Arsénico (As) es un elemento de gran toxicidad para el ser humano y su exposición prolongada, a través del consumo de agua y alimentos, puede causar cáncer y lesiones cutáneas. Las formas inorgánicas de arsénico (arsenato (As (V)) y arsenito (As (III))) son las más tóxicas, mientras que las formas metiladas (ácido monometilarsónico (MMA) y ácido dimetilarsónico (DMA)) son consideradas moderadamente tóxicas [1]. Otras especies de este elemento como la arsenobetaina (AsB) y los arsenoazúcares son considerados inocuas [2]. Por ello, es importante determinar qué especies de arsénico están presentes en biofluidos como la orina, ya proporcionan información sobre los mecanismos de detoxificación que se generan ante la presencia de este elemento y, por supuesto, las posibles repercusiones sanitarias derivadas del consumo de alimentos que contengan arsénico.

En el presente estudio se ha llevado a cabo la especiación analítica de As en 150 muestras de orinas procedentes de todo el territorio andaluz. Para ello, se ha utilizado la metodología propuesta por Contreras-Acuña et al [3], que de forma resumida se expone a continuación: dilución de la muestra de orina (1:5) con HNO<sub>3</sub> al 5% (v/v), separación de las especies de As presentes en el extracto (As (V), As (III), MA, DMA y AsB) mediante cromatografía líquida de intercambio aniónico (HPLC-AEC) acoplada a un detector ICP-MS. El procedimiento se ha validado utilizando un material de referencia ClinCheck® (level II) que contenía las especies estudiadas.

Los resultados muestran que las especies de As con mayor abundancia en las muestras de orinas son: AsB>DMA>As (V)>As (III)>MA, representando la concentración de AsB el 85% del As total. Este hecho es de gran interés ya que demuestra la ausencia de posibles problemas de salud pública derivados de la alimentación en la Comunidad Autónoma de Andalucía por el carácter inocuo de la AsB. Como detalles relevantes puede indicarse que la provincia andaluza cuya población presenta una mayor concentración de As total en la orina es Málaga y la de menor concentración Córdoba, posiblemente asociado al consumo de alimentos de origen marino.

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Los autores agradecen al Ministerio de Economía y Competitividad (España) la financiación del proyecto CTM2015-67902-C-1-P y a la Consejería de Innovación, Ciencia y Empresa (Junta de Andalucía) por el proyecto P012 FQM- 0442. Proyecto Exp. PI0530/2011 financiado por la Consejería de Salud. Los autores agradecen, a los fondos FEDER (Comunidad Europea) por la ayuda recibida proyectos UNHU13-1E-1611 y UNHU15-CE-3140.

## NON-CHROMATOGRAPHIC ARSENIC SPECIATION IN URINE

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The aim of this work is to propose a non-chromatographic method for determination of toxic arsenic species (As (III), As (V), MMA and DMA) in urine. Hydride generation coupled to atomic fluorescence spectrometry (HG-AFS) was employed as the experimental technique.

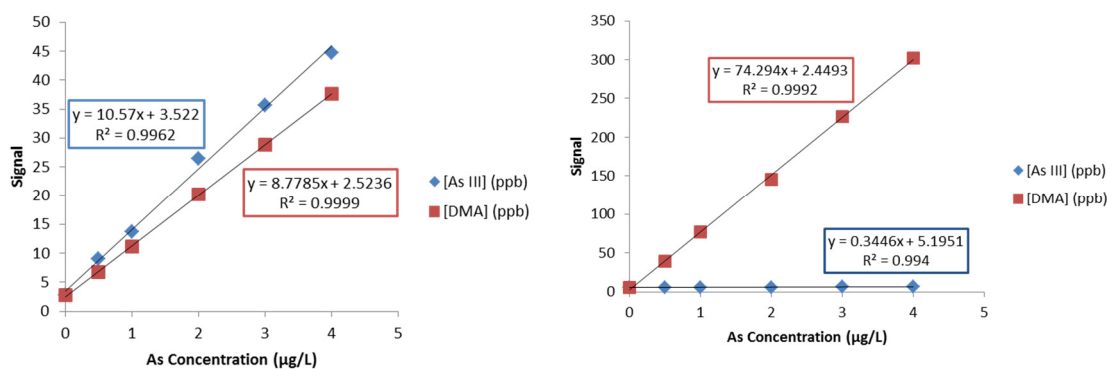
To attain these aim two strategies were assayed: i) Selection of the digestion conditions in which the four toxic arsenic species provide a comparable behaviour in front of hydride generation. ii) Study of hydride generation conditions under which the four species present the same sensitivity. In both cases it will be necessary to check that no signal was obtained from non toxic arsenic species, as arsenobetaine or arsenosugars.

Regarding the first strategy, it could be concluded that neither of the different digestion procedures assayed provided us similar behaviour for the four arsenic species in front of HG-AFS analysis.

The behaviour of the species in the analysis by HG-AFS depends on sodium borohydride reductor concentration, hydrochloric acid molarity and the type of pre-reductor employed. The inorganic species presents similar response in front of hydrochloric acid and sodium borohydride concentrations. And the same was observed for the both organic arsenic species.

The pre-reduction step affects all species very similarly, being observed fluorescence signals much higher when ascorbic acid and potassium iodide solutions were employed. There are two hydride generation conditions sets in which two species behave very similar to each other and so different from the other two that also have similar behaviour between them.

The accuracy of this method was evaluated by the percentage of recovery, varying between 84 to 110 %. On the other hand, the intake of fish products increases the toxic arsenic excreted in the urine.



Acknowledgements: Authors acknowledges the financial support from Generalitat Valenciana, project PROMETEO II 2014/077.

**CARACTERIZACIÓN DE ESPECIES DE PLATA LIBERADAS A PARTIR DE ARCILLAS RECUBIERTAS CON NANOPARTÍCULAS DE PLATA EN ENSAYOS DE DIGESTIBILIDAD MEDIANTE AF4-ICPMS**

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El uso tanto de la plata metálica como de sus sales en varias aplicaciones médicas o farmacéuticas debido a sus propiedades antimicrobianas es bien conocido. En cambio, su uso en alimentación animal como prebiótico no se ha desarrollado debido fundamentalmente al bajo coste de los antibióticos utilizados para mejorar el crecimiento. Tras la prohibición de esta práctica por la Unión Europea, los compuestos de plata se plantean ahora como una alternativa [1]. Las nanopartículas de plata (AgNPs) poseen un mayor efecto antimicrobiano respecto a las sales de plata, además de una menor absorción a través de la mucosa intestinal. Las arcillas se utilizan en alimentación animal para múltiples aplicaciones tecnológicas, nutricionales, sanitarias y ambientales. En este punto se plantea la utilización de sepiolitas y caolines, recubiertas con nanopartículas de plata como vehículos de administración de plata a los animales.

Se ha llevado a cabo la caracterización de las especies de plata liberada desde estos materiales en ensayos "in vitro" de simulación de las distintas etapas de digestión con el objetivo de estudiar las posibles transformaciones de la plata en estas condiciones y de entender los efectos del uso de estos materiales sobre el ecosistema microbiano digestivo. Esta caracterización se ha realizado mediante la técnica de Fraccionamiento en Flujo mediante Campo de Flujo Asimétrico (AF4) acoplado a un espectrómetro de masas con plasma de acoplamiento inductivo (ICPMS) como detector. Esta técnica permite detectar y caracterizar por tamaño las posibles nanopartículas de plata liberadas, la plata asociada a micropartículas de caolín o sepiolita, así como otras especies macromoleculares presentes en los medios complejos a los que se pueda asociar la plata. La fracción de plata iónica o asociada a especies de bajo peso molecular, que no es posible caracterizar mediante AF4, se aisló mediante ultrafiltración con membranas de pequeño tamaño de poro (3 kDa) y se cuantificó mediante ICPMS. El estudio del comportamiento de los materiales en medios de lixiviación simples (como agua ultrapura o HCl 0,01 M) ha permitido establecer la importancia de la composición del medio (portador) en el que se lleva a cabo la separación mediante AF4.

Este trabajo ha sido subvencionado por el proyecto CTQ2015-68094-C02 del Ministerio de Economía y Competitividad (MINECO) de España.

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**NEW PERSPECTIVES OF HYDRODYNAMIC CHROMATOGRAPHY  
COUPLED TO ICP-MS FOR SPECIATION ANALYSIS OF METALLIC  
NANOMATERIALS AND DISSOLVED METALS**

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Engineered nanoparticles (ENP) are increasingly being incorporated into consumer products and reaching the environment at a growing rate. This fact requires the adaptation of existing techniques and methods, or the development of new ones to monitor their occurrence, fate and transformations. In order to understand the environmental impact of ENP, it is critical to discriminate among dissolved and particulate forms. Different techniques and methodological approaches for the characterization and quantification of ENP and its derivatives in complex samples have been recently reviewed [1].

Asymmetric flow field flow fractionation (AF4) and hydrodynamic chromatography (HDC) are commonly coupled to ICP-MS as element specific detector for the separation and determination of inorganic ENP in a variety of samples. Although HDC is a robust and versatile separation technique, its resolving power is much lower than AF4. However, separations take place in a shorter time and dissolved low molecular mass species are not lost, as in AF4 due to the ultrafiltration membranes used in its separation channel. Thus HDC-ICP-MS can provide simultaneous information about dissolved and particulate species of an element in less than about ten minutes, which is not the case for AF4.

Most applications of HDC-ICP-MS involve the use of the commercially available PL-PSDA HDC column and a proprietary mobile phase, comprised of a salt mixture and surfactants. In this work, chromatographic parameters as well as mobile phase compositions have been systematically studied with the column cited above, to evaluate the performance of HDC-ICP-MS for the simultaneous determination of dissolved species and nanoparticles of the same element. Special attention was paid to the resolution achieved between dissolved species and nanoparticles and their column recovery.

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This work was supported by the Spanish Ministry of Economy and Competitiveness, project CTQ2015-68094-C2-1-R.

**CHARACTERIZATION OF METALS PROFILES IN SERUM, URINE AND  
BRONCHOALVEOLAR LAVAGE FLUID FROM LUNG CANCER  
PATIENTS USING ICP-QQQ-MS.**

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Lung cancer (LC) is one of the most common causes of cancer-related deaths on the world [1,2], and it is well known that trace elements play an essential role in a number of biological processes by activating or inhibiting enzymatic reactions. Therefore, involvement of metals in the carcinogenic process is of great interest [3]. To this end, an analytical method based on ICP-QQQ-MS was optimized for eleven elements: V, Cr, Mn, Fe, Co, Cu, Zn, Se, Mo, Cd, and Pb in order to characterize the metal profile in biological fluids of patient with lung cancer. Some of these elements are considered toxic, such as V, Cd, Cr and Pb, whereas Co, Mo, Se, Fe and Zn are essential. Cu and Zn have been associated to blood pressure regulation and their imbalance has been related to hypercholesterolemia, arterial damage and myocardial infarction [4]. However, selenium has lung anticancer properties [5]. In addition, an analytical approach based on non-denaturing precipitation of proteins has been optimized for the fractionation of high molecular mass (HMM) and low molecular mass (LMM) metal species from serum to distinguish between species because it affects biological activity or toxicological potential of the element.

There are a lot of studies about the determination of trace elements in blood, urine and lung tissues in relation to the metal distribution in lung cancer patients, but only a few are referred to bronchoalveolar lavage fluid (BALF), and the combined information about different fluids has to be still established. Given that BALF provides information on cellular and biochemical epithelial surface of the lower respiratory tract constituents [6], the correlation with peripheral fluids as serum is of interest.

In this work, multi-elemental determination in serum, urine and BALF samples using ICP-QQQ-MS was performed to know the metal distribution in LC samples. In addition, control samples (C) were analysed to compare with LC and establish differences between them in order to find some elements which could serve as biomarkers. On the other hand, statistical analysis based on Partial Least Square Discriminant Analysis (PLS-DA) was used to classify controls and LC groups, and Mann-Whitney U test was carried out to establish significant differences between LC and control groups. Finally, correlation analysis (Spearman's test) indicated that these metal abnormalities can be interrelated, participating in common processes such as oxidative stress and altered homeostasis.

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**Acknowledgements:** This work was supported by the projects CTM2012-38720-C03-01 from the Spanish Ministry of Economy and Competitiveness and P12-FQM-0442 from the Regional Ministry of Economy, Innovation, Science and Employment (Andalusian Government, Spain).



**ESTUDIO DE LA HOMEOSTASIS DE METALES Y METABOLITOS RELACIONADOS CON LA EXPOSICIÓN A METALES (ARSÉNICO, CADMIO Y MERCURIO) EN EL RATÓN *MUS MUSCULUS* MEDIANTE TÉCNICAS METALO-METABOLÓMICAS. ACCIÓN ANTAGONISTA CON EL SELENIO.**

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Los metales tienen un papel fundamental en los sistemas biológicos, regulando numerosos procesos celulares y, alternativamente, desarrollando efectos tóxicos sobre el metabolismo [1]. Por lo tanto, el estudio de los cambios inducidos por los metales en el metabolismo celular es crucial para comprender la respuesta biológica asociada a la presencia de contaminantes.. Arsénico (As), Cadmio (Cd) y Mercurio (Hg) son metales tóxicos de importancia ambiental con efectos perjudiciales para el hombre. Además, se conoce que el Selenio (Se) presenta interacciones antagónicas con numerosos elementos [2]. En el presente trabajo, el ratón *Mus musculus* fue expuesto durante un periodo de 10 días a la acción de estos elementos: As, en forma de NaAsO<sub>2</sub>; Cd, en forma de CdCl<sub>2</sub>; y Hg como HgCl<sub>2</sub>. Además, en este estudio se evaluaron las interacciones antagonistas de estos elementos con Se. Las concentraciones totales de metales fueron analizadas por ICP-QQQ-MS en diferentes tejidos: hígado, riñón, corazón, testículo, cerebro y suero sanguíneo para evaluar el impacto de estos contaminantes sobre la homeostasis global en los organismos vivos. Complementariamente, los cambios en el metabolismo inducido por esta mezcla de metales se evaluaron en suero mediante metabolómica no dirigida [3].

Los resultados muestran información sobre la distribución de los elementos, sus interacciones, homeostasis y perturbación metabólica. Además, revelan el potencial del uso combinado de técnicas metalómicas y metabolómicas en los experimentos de exposición ambiental.

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**NOVEL GC BASED STRATEGIES FOR THE ACCURATE AND SENSITIVE SPECIATION OF SO<sub>2</sub> IN WINE****Vanessa Carrascón, Ignacio Ontañón, Mónica Bueno, Vicente Ferreira**

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Sulfur dioxide has been widely used in the food industry because of its antioxidant, antioxidasic and antiseptic properties. SO<sub>2</sub> may appear in wine under different species due to its acid-base properties and to the more or less reversible adducts that it can form with acetaldehyde, sugars, polyphenols and other carbonyls. Speciation of this molecule is essential because its bioactive and antioxidant activities are extremely dependent on the specific chemical species. Total levels of SO<sub>2</sub> are important because of safety and legal reasons. Bound sulfur dioxide represents a complex pool of diverse molecules from which free SO<sub>2</sub> can be released. Such release, inevitably will have consequences on wine sensory properties, since the cleavage of SO<sub>2</sub> adducts will release anthocyanins, and sensory relevant aldehydes. Bisulfite (HSO<sub>3</sub><sup>-</sup>) is important due to its antioxidant and antioxidasic properties and molecular SO<sub>2</sub> (SO<sub>2</sub> plus H<sub>2</sub>SO<sub>3</sub>) is the most bioactive form and the main responsible for microbial stability. The reference method of aspiration/titration for determining free SO<sub>2</sub> fails when levels drop below 7-8 mg/L. Moreover, this method is based on an unspecific determination in which acid volatile compounds can produce interferences which can be important at low levels. Additionally, there are doubts about whether the free SO<sub>2</sub> determined by the reference method is all equally active, because it has been shown that wines with similar levels of SO<sub>2</sub> have different level of protection [1].

A three determination strategy allowing to quantify three biologically and technologically-relevant SO<sub>2</sub>-categories has been developed for the speciation of SO<sub>2</sub>. Determinations are in all cases based on the GC-MS or GC-SCD (sulfur chemiluminescent detector) analyses of the vapors on the headspaces.

- 1) Total forms are determined by headspace analysis of the acidified sample after incubation at 100°C
- 2) Free and weakly-bound SO<sub>2</sub>, which from here will be named as “nominally freeSO<sub>2</sub>” is similarly determined by headspace analysis of the acidified sample, but incubation is carried out at 40°C
- 3) Truly free (or molecular) SO<sub>2</sub> is finally determined well in the headspace of an acidified fraction obtained by purging the wine with nitrogen and trapping SO<sub>2</sub> in an aqueous alkaline solution (pH 11.5), well directly in the headspace of the unaltered wine if the more sensitive GC-SCD is available

Results for total and nominally free fractions are statistically comparable to those provided by the reference method, but the strategies are much more sensitive (LD improved by factors up to 10) and reproducible (ca. 3.5%). Regarding the truly free fraction, results have demonstrated that between 10 and 80% of the free SO<sub>2</sub> measured by the reference method is in fact forming complexes with polyphenols which are cleaved during the analysis, which could have relevant technological consequences.

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